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T|N

PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

Date of mailing (day/month/year) 03 January 2000 (03.01.00)	
International application No. PCT/EP99/03255	**Applicant's or agent's file reference** FB/BM45321
International filing date (day/month/year) 07 May 1999 (07.05.99)	**Priority date** (day/month/year) 13 May 1998 (13.05.98)
Applicant RUELLE, Jean-Louis	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

30 November 1999 (30.11.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Jean-Marie McAdams
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

T/N

PATENT COOPERATION TREATY

PCT/EP99/03255

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark
Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

In its capacity as elected Office

Date of mailing (day/month/year)	
03 January 2000 (03.01.00)	
International application No.	Applicant's or agent's file reference
PCT/EP99/03255	FB/BM45321
International filing date (day/month/year)	Priority date (day/month/year)
07 May 1999 (07.05.99)	13 May 1998 (13.05.98)
Applicant	
RUELLE, Jean-Louis	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

30 November 1999 (30.11.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Jean-Marie McAdams
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Copy for the elected Office (EO/US)

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

COMMUNICATION IN CASES FOR WHICH NO OTHER FORM IS APPLICABLE

To:
Kathryn PRIVETT
SmithKline Beecham
Two New Horizons Court
Brentford
Middlesex TW8 9EP
Royaume-Uni

Date of mailing (*day/month/year*) 18 December 2000 (18.12.00)	
Applicant's or agent's file reference FB/BM45321	**REPLY DUE** see paragraph 1 below
International application No. PCT/EP99/03255	International filing date (*day/month/year*) 07 May 1999 (07.05.99)
Applicant SMITHKLINE BEECHAM BIOLOGICALS S.A.	

1. ☐ REPLY DUE within _____ months/days from the above date of mailing

☐ NO REPLY DUE, however, see below

☒ IMPORTANT COMMUNICATION

☐ INFORMATION ONLY

2. COMMUNICATION:

It has been brought to the attention of the International Bureau that in respect of the above-identified application, the 10 pages of sequence listing were erroneously omitted in the international publication N° WO99/58683 on 18 November 1999 (18.11.99).

The International Bureau shall publish a correction in Section II of the PCT Gazette. A corrected version of the corresponding PCT pamphlet will be published on that same date.

A copy of this notification is being sent to the receiving Office (RO/EP) and to the elected Offices concerned.

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Christine Carrié
Facsimile No. (41-22) 740.14.35	Telephone No. (41-22) 338.83.38

For receiving Office use only 09/700293

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty

International Application No:

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired)(12 characters maximum)* FB/BM45321

Box No. I TITLE OF INVENTION NOVEL COMPOUNDS

Box No. II APPLICANT

Name and Address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country)*

SmithKline Beecham Biologicals s.a.
Rue de l'Institut 89
B1330 Rixensart
BE

☐ This person is also inventor

Telephone No.

Facsimile No.

Teleprinter No.

State *(i.e. country)* of nationality: BE

State *(i.e. country)* of residence: BE

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental box

Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS

Name and Address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country)*

RUELLE, Jean-Louis
SmithKline Beecham Biologicals s.a.
Rue de l'Institut 89
B1330 Rixensart
BE

☐ This person applicant only

☒ applicant and inventor

☐ inventor only *(if this check-box is marked, do not fill in below)*

State *(i.e. country)* of nationality: BE

State *(i.e. country)* of residence: BE

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental box

☐ Further applicants and/or (further) inventors are indicated on a continuation sheet

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and Address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country)*

TYRRELL, Arthur William Russell
SmithKline Beecham
Two New Horizons Court
Brentford
Middlesex
TW8 9EP
GB

Telephone No +44 181 975 6349

Facsimile No.

+44 181 975 6294

Teleprinter No.

☐ Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent

Box No. V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes: at least one must be marked)*:

Regional Patent

☒ AP ARIPO Patent: GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SZ Swaziland, UG Uganda, ZW Zimbabwe and any other state which is a Contracting State of the Harare Protocol and of the PCT

☒ EA Eurasian Patent: AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgystan, KZ Kazakstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT

☒ EP European Patent: AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT

☒ OA OAPI Patent: BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT (if other kind of protection or treatment desired, specify on dotted line).....

National Patent (if other kind of protection or treatment desired, specify on dotted line):

☒ AL Albania
☒ AM Armenia
☒ AT Austria
☒ AU Australia
☒ AZ Azerbaijan
☒ BA Bosnia and Herzegovina
☒ BB Barbados
☒ BG Bulgaria
☒ BR Brazil
☒ BY Belarus
☒ CA Canada
☒ CH and LI Switzerland and Liechtenstein
☒ CN China
☒ CU Cuba
☒ CZ Czech Republic
☒ DE Germany
☒ DK Denmark
☒ EE Estonia
☒ ES Spain
☒ FI Finland
☒ GB United Kingdom
☒ GE Georgia
☒ GH Ghana
☒ GM Gambia
☒ GW Guinea-Bissau
☒ HR Croatia
☒ HU Hungary
☒ ID Indonesia
☒ IL Israel
☒ IS Iceland
☒ JP Japan
☒ KE Kenya
☒ KG Kyrgyzstan
☒ KP Democratic People's Republic of Korea
☒ KR Republic of Korea
☒ KZ Kazakstan
☒ LC St Lucia
☒ LK Sri Lanka
☒ LR Liberia
☒ LS Lesotho
☒ LT Lithuania
☒ LU Luxembourg
☒ LV Latvia
☒ MD Republic of Moldova
☒ MG Madagascar
☒ MK The former Yugoslav Republic of Macedonia
☒ MN Mongolia
☒ MW Malawi
☒ MX Mexico
☒ NO Norway
☒ NZ New Zealand
☒ PL Poland
☒ PT Portugal
☒ RO Romania
☒ RU Russian Federation
☒ SD Sudan
☒ SE Sweden
☒ SG Singapore
☒ SI Slovenia
☒ SK Slovakia
☒ SL Sierra Leone
☒ TJ Tajikistan
☒ TM Turkmenistan
☒ TR Turkey
☒ TT Trinidad and Tobago
☒ UA Ukraine
☒ UG Uganda
☒ US United States of America
☒ UZ Uzbekistan
☒ VN Viet Nam
☒ YU Yugoslavia
☒ ZW Zimbabwe

Check boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

☒ and all States which have become party to the PCT after issuance of this sheet
☒ IN India
☒ GD Grenada
☒ ZA South Africa
☒ AE United Arab Emirates

In addition to the designations made above, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except the designation(s) of: none. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Box No. VI PRIORITY CLAIM Further priority claims are indicated in the Supplemental Box ☐

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1) 13 May 1998 (13/05/98)	9810276.7	GB		
item (2)				
item (3)				

☐ The receiving Office is hereby requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving office)* identified above as item(s): _____

**Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which the earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA)

(If two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):

ISA/ _____

Request to use results of earlier search; reference to that search (if an earlier search has been carried out by or requested from the International Searching Authority):

Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following number of sheets:

request	: 3
description (excluding sequence listing part)	: 56
claims	: 4
abstract	: 1
drawings	: 12
sequence listing part of description	: 10
Total number of sheets	**: 86**

This international application is accompanied by the item(s) marked below:

1. ☒ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☒ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☒ priority document(s) identified in Box No.VI as item(s): 1
6. ☐ translation of international application into (language):
7. ☒ separate indications concerning deposited microorganism or other biological material
8. ☒ nucleotide and/or amino acid sequence listing in computer readable form
9. ☐ other *(specify)*

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: English

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such is not obvious from reading the request).

TYRRELL, Arthur William Russell
Agent for the Applicant

For receiving Office use only

1.	Date of actual receipt of the purported international application		2. Drawings:
3.	Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:		☐ received:
4.	Date of timely receipt of the required corrections under PCT Article 11(2)		☐ not received:
5.	International Searching Authority (if two or more are competent) ISA/	6. ☐ Transmittal of search copy delayed until search fee is paid	

For International Bureau use only

Date of receipt of the record copy by the International Bureau

09/11 '00 15:18 FAX 0208 975 6294 SB CORP IP

09/700293

EUROPÄISCHES PATENTAMT
Generaldirektion 1

EUROPEAN PATENT OFFICE
Directorate General 1

OFFICE EUROPÉEN DES BREVETS
Direction générale 1

Patentlaan 2
2280 HV Rijswijk ZH
Nederland
Tel 070-3402040
Telex 31651
Fax 070-3403016

Tyrrell, Arthur W.R.
SMITHKLINE BEECHAM
Corporate Intellectual Property
Two New Horizons Court
Brentford
Middlesex TW8 9EP
ENGLAND

RECEIVED
17 MAY 1999
NEW HORIZONS COURT

Nr. der Anmeldung - Application N° - Demande de brevet n°	PCT/EP 99/03255
Tag des Eingangs - Date of Receipt - Date de réception	07 MAY 1999
Aktenzeichen des Anmelders/Anwalts - Applicant's/Agent's file reference - Coté du dossier du déposant ou du mandataire	FB/BM45321

Datum / Date 14.05.99

Betreff: Empfangsbestätigung
Re: Receipt for documents
Objet: Récépissé de documents

Das Europäische Patentamt
The European Patent Office
L'Office européen des brevets

bestätigt hiermit den Empfang folgender Dokumente:
hereby acknowledges the receipt of the following:
accuse réception des documents indiqués ci-dessous:

A. Anmeldeunterlagen / items making up the application / pièces de la demande	Anzahl / N° of copies / Nombre d'exemplaires
[X] Antrag / Request / Requête	1
[X] Beschreibung / Description	3
[X] Ansprüche / Claim(s) / Revendication(s)	3
[X] Zeichnung(en) / Drawing(s) / Dessin(s)	3
[X] Zusammenfassung / Abstract / Abrégé	3

B. Beigefügte Dokumente / accompanying documents / documents joints	
[X] Vertretervollmacht / Authorisation of representative(s) / Pouvoir de mandataire	
[X] Prioritätsdokument(e) / Priority document(s) / Document(s) de priorité	
[ ] Scheck / Cheque / Chèque	
[X] Antrag zur Belastung des laufenden Konto / Request to charge deposit account / Demande de débit de compte-courant	
[X] Andere Unterlagen / Other Documents / Autres documents	sequence listing diskette

Die genannten Unterlagen sind am obengenannten Tag eingegangen; die Anmeldung hat die ebenfalls oben angeführte Anmeldenummer erhalten.

The said items were received on the date indicated above and the application has been assigned the above indicated application number.

Les documents mentionnés ont été reçus à la date indiquée ci-dessus et le numéro de demande de brevet indiqué ci-dessus a été attribué à la demande.

ON DATABASE
27 MAY 1999
PCM WIN SOURCE M

Unterschrift/Amtsstempel
Signature/Official Stamp
Signature/Cachet officiel

M. DAVIDSON

PATENT COOPERATION TREATY

09/700293

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

Kathryn Louise PRIVETT
SMITHKLINE BEECHAM PLC
Corporate Intellectual Property
Two New Horizons Court
Brentford
Middlesex TW8 9EP
GRANDE BRETAGNE

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	06.09.00
Applicant's or agent's file reference KP/DM/BM45321	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP99/03255	07/05/1999	13/05/1998

Applicant
SMITHKLINE BEECHAM BIOLOGICALS S.A.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Vullo, C Tel.+49 89 2399-8061

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
KP/DM/BM45321		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP99/03255	07/05/1999	13/05/1998
International Patent Classification (IPC) or national classification and IPC C12N15/31		
Applicant SMITHKLINE BEECHAM BIOLOGICALS S.A.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
30/11/1999	06.09.00
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Page, M Telephone No. +49 89 2399 7322

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP99/03255

I. Basis of th report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-55	as originally filed

Claims, No.:

1-25	as received on	09/08/2000	with letter of	09/08/2000

Drawings, sheets:

1/12-12/12	as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-4,7-17,24
	No:	Claims	5,6,18-20-23,25
Inventive step (IS)	Yes:	Claims	1-4,7-17,24
	No:	Claims	5,6,18-23,25
Industrial applicability (IA)	Yes:	Claims	1-25
	No:	Claims	

2. Citations and explanations

see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item I

Basis of the report

The examination is being carried out on the **following application documents**:

Text for the Contracting States:
AT BE CH DE DK ES FI FR GB GR IT IE LI LU MC NL PT SE

Description, pages:

1-55	as originally filed				

Claims, No.:

1-25	as received on	09/08/2000	with letter of	09/08/2000	

Drawings, sheets:

1/12-12/12	as originally filed

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1) Reference is made to the following documents:

D1: ST GEME JW 3RD, CUTTER D, BARENKAMP SJ : 'Characterization of the genetic locus encoding Haemophilus influenzae type b surface fibrils' J BACTERIOL. , vol. 178, no. 21, November 1996 (1996-11), pages 6281-6287, XP000863110

D2: EP-A-0 301 992 (NACIONAL DE BIOPREPARADOS CENT) 1 February 1989 (1989-02-01)

D3: WO 93 06861 A (PASTEUR MERIEUX SERUMS VACC) 15 April 1993 (1993-04-15)

2) **Novelty - Art.33(1) and (2) PCT:**

Claims 1-4 are novel in the light of the prior art. Although D1 discloses a mix of potentially antigenic proteins that contains the proteins of the present application, the applicant's argument that the claimed isolated polypeptides corresponding to SEQ ID NOs. 2 and 4 are novel is followed.

Claims 5 and 6 cannot be considered as being novel in the light of the prior art. A "peptide comprising an immunogenic fragment" is considered to include any hexameric peptide of SEQ ID NOs. 2 or 4 that can be used as an antigen in the raising of an immune response which would recognise the said polypeptides. Without reference to further sequences, it is considered that not all hexamers of the present application are novel and that therefore the subject matter of claims 5 and 6 is not novel. See further Item VIII a) *infra.*

Claims 7-17, 19 and 24 appear to be novel in the light of the prior art. Polynucleotides encoding the claimed polypeptides have not been previously disclosed and therefore neither has their use in the expression of the claimed polypeptides.

Claims 18-20 and 23 lack novelty in light of the disclosure of D1 regarding a vaccine comprising a protein antigenic complex (D1 claim 11). It cannot be seen how the subject matter of the present application differs from the prior art at this point, as both can be considered to be a 'vaccine composition *comprising* an effective amount of the polypeptide' of the application.

Claims 21 and 25 lack novelty in light of the subject matter of D1 claim 13 concerning an antimeningococcic hyperimmune gammaglobulin preparation, which is considered to include antibodies against the polypeptide of the present application.

Furthermore, claims 18, 20-23 and 25 cannot be considered novel due to their dependence on claims 5 and 6 (*supra*). Claim 21 is further considered to lack novelty in the light of D1, which discloses antibodies against a mix of antigenic proteins (D1 claim 13). The mix is considered to contain proteins of the present application and thus the subject matter of the present claim 21 cannot be distinguished from previously disclosed subject matter. Had the subject matter of these claims been

restricted to dependency on claims 1-4, it would have been possible to acknowledge novelty.

3) **Inventive Step - Art.33(1) and (3) PCT:**

The following comments on inventive step are confined to subject matter which could be acknowledged as being novel.

The closest prior art is document D3, which discloses polynucleotide sequences encoding N.m. membrane-bound polypeptides and the subsequent use of these polypeptides in the preparation of a vaccine against the said organism.
The objective technical problem can be seen as being the provision of alternative polynucleotide and polypeptide sequences for the purpose of preparing a vaccine against N.m. infection, as well as the diagnosis of said infection using either the said polypeptides or antibodies generated against these peptides.

The technical problem is solved by the subject matter of claims 1-25, which provide polynucleotide and polypeptide sequences corresponding to a 65 kDa N.m. surface protein.

Claims 1-4, 7-17 and 24 can be acknowledged as demonstrating inventive step. Although the prior art discloses sequence information for other putative antigens, no suggestion is made that the subject matter disclosed in the present application would also fulfill this role.

Re Item VI
Certain documents cited

Certain published documents (Rule 70.10)

Application No Patent No	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date *(valid claim)* *(day/month/year)*
WO 99 31132 A	24.06.1999	14.12.1998	12.12.1997

Re Item VII
Certain defects in the international application

a) Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1-D3 are not mentioned in the description, nor are these documents identified therein.

Re Item VIII
Certain observations on the international application

a) The term "polypeptide comprising an immunogenic fragment" (page 5 lines 4-7 and claims 5 and 6) is considered to include every hexameric peptide of the application and therefore renders the scope of the said claims obscure (Article 6 PCT).

b) The term "isolated polypeptide" in claims 1, 2 and 4 is ambiguous rendering the scope of the said claims obscure (thus contravening Article 6 PCT). Particularly in claim 4, it is unclear whether "isolated polypeptide" is intended to mean a portion of the disclosed polypeptide sequence or the whole polypeptide sequence. For the purposes of examination, the latter has been assumed.

c) Further terms in the description that render the scope of the claimed subject matter obscure include "structural fragments" (page 5 line 27 - page 6 line 3), "polypeptides comprising an amino acid sequence having at least... ...contiguous amino acids" (page 6 lines 6 and 8), polypeptide variants in which "amino acids are substituted, deleted or added in any combination" (page 6 lines 16-17), "fragment thereof" (page 22 lines 1-2) and "epitope-bearing fragments" (page 24 lines 25-26). These expressions should be more precisely defined in their description of the subject matter being claimed (Art. 6 PCT).

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(54) Title: BASB029 POLYNUCLEOTIDE(S) AND POLYPEPTIDES FROM *NEISSERIA MENINGITIDIS*

(57) Abstract: The invention provides BASB029 polypeptides and polynucleotides encoding BASB029 polypeptides and methods for producing such polypeptides by recombinant techniques. Also provided are diagnostic, prophylactic and therapeutic uses.

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BASB029 POLYNUCLEOTIDE(S) AND POLYPEPTIDES FROM *NEISSERIA MENINGITIDIS*

FIELD OF THE INVENTION

This invention relates to polynucleotides, (herein referred to as "BASB029 polynucleotide(s)"), polypeptides encoded by them (referred to herein as "BASB029" or "BASB029 polypeptide(s)"), recombinant materials and methods for their production. In another aspect, the invention relates to methods for using such polypeptides and polynucleotides, including vaccines against bacterial infections. In a further aspect, the invention relates to diagnostic assays for detecting infection of certain pathogens.

BACKGROUND OF THE INVENTION

Neisseria meningitidis (meningococcus) is a Gram-negative bacterium frequently isolated from the human upper respiratory tract. It occasionally causes invasive bacterial diseases such as bacteremia and meningitis. The incidence of meningococcal disease shows geographical seasonal and annual differences (Schwartz, B., Moore, P.S., Broome, C.V.; Clin. Microbiol. Rev. 2 (Supplement), S18-S24, 1989). Most disease in temperate countries is due to strains of serogroup B and varies in incidence from 1-10/100,000/year total population sometimes reaching higher values (Kaczmarski, E.B. (1997), Commun. Dis. Rep. Rev. 7: R55-9, 1995; Scholten, R.J.P.M., Bijlmer, H.A., Poolman, J.T. et al. Clin. Infect. Dis. 16: 237-246, 1993; Cruz, C., Pavez, G., Aguilar, E., et al. Epidemiol. Infect. 105: 119-126, 1990).

Epidemics dominated by serogroup A meningococci, mostly in central Africa, are encountered, sometimes reaching levels up to 1000/100.000/year (Schwartz, B., Moore, P.S., Broome, C.V. Clin. Microbiol. Rev. 2 (Supplement), S18-S24, 1989). Nearly all cases as a whole of meningococcal disease are caused by serogroup A, B, C, W-135 and Y meningococci and a tetravalent A, C, W-135, Y polysaccharide vaccine is available (Armand, J., Arminjon, F., Mynard, M.C., Lafaix, C., J. Biol. Stand. 10: 335-339, 1982).

The polysaccharide vaccines are currently being improved by way of chemical conjugating them to carrier proteins (Lieberman, J.M., Chiu, S.S., Wong, V.K., et al. JAMA 275 : 1499-1503, 1996).

A serogroup B vaccine is not available, since the B capsular polysaccharide was found to be nonimmunogenic, most likely because it shares structural similarity to host components (Wyle, F.A., Artenstein, M.S., Brandt, M.L. et al. J. Infect. Dis. 126: 514-522, 1972; Finne, J.M., Leinonen, M., Mäkelä, P.M. Lancet ii.: 355-357, 1983).

For many years efforts have been initiated and carried out to develop meningococcal outer membrane based vaccines (de Moraes, J.C., Perkins, B., Camargo, M.C. et al. Lancet 340: 1074-1078, 1992; Bjune, G., Hoiby, E.A. Gronnesby, J.K. et al. 338: 1093-1096, 1991). Such vaccines have demonstrated efficacies from 57% - 85% in older children (>4 years) and adolescents.

Many bacterial outer membrane components are present in these vaccines, such as PorA, PorB, Rmp, Opc, Opa, FrpB and the contribution of these components to the observed protection still needs futher definition. Other bacterial outer membrane components have been defined by using animal or human antibodies to be potentially relevant to the induction of protective immunity, such as TbpB and NspA (Martin, D., Cadieux, N., Hamel, J., Brodeux, B.R., J. Exp. Med. 185: 1173-1183, 1997; Lissolo, L., Maître-Wilmotte, C., Dumas, p. et al., Inf. Immun. 63: 884-890, 1995). The mechanisms of protective immunity will involve antibody mediated bactericidal activity and opsonophagocytosis.

A bacteremia animal model has been used to combine all antibody mediated mechanisms (Saukkonen, K., Leinonen, M., Abdillahi, H. Poolman, J. T. Vaccine 7: 325-328, 1989). It is generally accepted that the late complement component mediated bactericidal mechanism is

crucial for immunity against meningococcal disease (Ross, S.C., Rosenthal P.J., Berberic, H.M., Densen, P. J. Infect. Dis. 155: 1266-1275, 1987).

The frequency of *Neisseria meningitidis* infections has risen dramatically in the past few decades. This has been attributed to the emergence of multiply antibiotic resistant strains and an increasing population of people with weakened immune systems. It is no longer uncommon to isolate *Neisseria meningitidis* strains that are resistant to some or all of the standard antibiotics. This phenomenon has created an unmet medical need and demand for new anti-microbial agents, vaccines, drug screening methods, and diagnostic tests for this organism.

SUMMARY OF THE INVENTION

The present invention relates to BASB029, in particular BASB029 polypeptides and BASB029 polynucleotides, recombinant materials and methods for their production. In another aspect, the invention relates to methods for using such polypeptides and polynucleotides, including prevention and treatment of microbial diseases, amongst others. In a further aspect, the invention relates to diagnostic assays for detecting diseases associated with microbial infections and conditions associated with such infections, such as assays for detecting expression or activity of BASB029 polynucleotides or polypeptides.

Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following descriptions and from reading the other parts of the present disclosure.

DESCRIPTION OF THE INVENTION

The invention relates to BASB029 polypeptides and polynucleotides as described in greater detail below. In particular, the invention relates to polypeptides and polynucleotides of BASB029 of *Neisseria meningitidis*, which is related by amino acid sequence homology to *Haemophilus influenzae* surface fibril (HSF) protein. The invention relates especially to BASB029 having the nucleotide and amino acid sequences set out in SEQ ID NO:1,3 and SEQ ID NO:2,4 respectively. It is understood that sequences recited in the Sequence Listing below as "DNA" represent an exemplification of one embodiment of the invention, since those of ordinary skill will recognize that such sequences can be usefully employed in polynucleotides in general, including ribopolynucleotides.

Polypeptides

In one aspect of the invention there are provided polypeptides of *Neisseria meningitidis* referred to herein as "BASB029" and "BASB029 polypeptides" as well as biologically, diagnostically, prophylactically, clinically or therapeutically useful variants thereof, and compositions comprising the same.

The present invention further provides for:

(a) an isolated polypeptide which comprises an amino acid sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% or exact identity, to that of SEQ ID NO:2, 4;

(b) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:1, 3 over the entire length of SEQ ID NO:1, 3 respectively; or

(c) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity, to the amino acid sequence of SEQ ID NO:2, 4;

The BASB029 polypeptides provided in SEQ ID NO:2,4 are the BASB029 polypeptides from *Neisseria meningitidis* strains ATCC13090 and H44/76.

The invention also provides an immunogenic fragment of a BASB029 polypeptide, that is, a contiguous portion of the BASB029 polypeptide which has the same or substantially the same immunogenic activity as the polypeptide comprising the amino acid sequence of SEQ ID NO:2,4. That is to say, the fragment (if necessary when coupled to a carrier) is capable of raising an immune response which recognises the BASB029 polypeptide. Such an immunogenic fragment may include, for example, the BASB029 polypeptide lacking an N-terminal leader sequence, and/or a transmembrane domain and/or a C-terminal anchor domain. In a preferred aspect the immunogenic fragment of BASB029 according to the invention comprises substantially all of the extracellular domain of a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% identity, to that of SEQ ID NO:2,4 over the entire length of SEQ ID NO:2

A fragment is a polypeptide having an amino acid sequence that is entirely the same as part but not all of any amino acid sequence of any polypeptide of the invention. As with BASB029 polypeptides, fragments may be "free-standing," or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region in a single larger polypeptide.

Preferred fragments include, for example, truncation polypeptides having a portion of an amino acid sequence of SEQ ID NO:2,4 or of variants thereof, such as a continuous series of residues that includes an amino- and/or carboxyl-terminal amino acid sequence. Degradation forms of the polypeptides of the invention produced by or in a host cell, are also preferred. Further preferred are fragments characterized by structural or functional attributes such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-

forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions.

Further preferred fragments include an isolated polypeptide comprising an amino acid sequence having at least 15, 20, 30, 40, 50 or 100 contiguous amino acids from the amino acid sequence of SEQ ID NO:2,4, or an isolated polypeptide comprising an amino acid sequence having at least 15, 20, 30, 40, 50 or 100 contiguous amino acids truncated or deleted from the amino acid sequence of SEQ ID NO:2,4.

Fragments of the polypeptides of the invention may be employed for producing the corresponding full-length polypeptide by peptide synthesis; therefore, these fragments may be employed as intermediates for producing the full-length polypeptides of the invention.

Particularly preferred are variants in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acids are substituted, deleted, or added in any combination.

The polypeptides, or immunogenic fragments, of the invention may be in the form of the "mature" protein or may be a part of a larger protein such as a precursor or a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, or an additional sequence for stability during recombinant production. Furthermore, addition of exogenous polypeptide or lipid tail or polynucleotide sequences to increase the immunogenic potential of the final molecule is also considered.

In one aspect, the invention relates to genetically engineered soluble fusion proteins comprising a polypeptide of the present invention, or a fragment thereof, and various

portions of the constant regions of heavy or light chains of immunoglobulins of various subclasses (IgG, IgM, IgA, IgE). Preferred as an immunoglobulin is the constant part of the heavy chain of human IgG, particularly IgG1, where fusion takes place at the hinge region. In a particular embodiment, the Fc part can be removed simply by incorporation of a cleavage sequence which can be cleaved with blood clotting factor Xa.

Furthermore, this invention relates to processes for the preparation of these fusion proteins by genetic engineering, and to the use thereof for drug screening, diagnosis and therapy. A further aspect of the invention also relates to polynucleotides encoding such fusion proteins. Examples of fusion protein technology can be found in International Patent Application Nos. WO94/29458 and WO94/22914.

The proteins may be chemically conjugated, or expressed as recombinant fusion proteins allowing increased levels to be produced in an expression system as compared to non-fused protein. The fusion partner may assist in providing T helper epitopes (immunological fusion partner), preferably T helper epitopes recognised by humans, or assist in expressing the protein (expression enhancer) at higher yields than the native recombinant protein. Preferably the fusion partner will be both an immunological fusion partner and expression enhancing partner.

Fusion partners include protein D from *Haemophilus influenzae* and the non-structural protein from influenzae virus, NS1 (hemagglutinin). Another fusion partner is the protein known as LytA. Preferably the C terminal portion of the molecule is used. LytA is derived from *Streptococcus pneumoniae* which synthesize an N-acetyl-L-alanine amidase, amidase LytA, (coded by the lytA gene {Gene, 43 (1986) page 265-272}) an autolysin that specifically degrades certain bonds in the peptidoglycan backbone. The C-terminal domain of the LytA protein is responsible for the affinity to the choline or to some choline analogues such as DEAE. This property has been exploited for the development of *E.coli* C-LytA expressing plasmids useful for

expression of fusion proteins. Purification of hybrid proteins containing the C-LytA fragment at its amino terminus has been described {Biotechnology: 10, (1992) page 795-798}. It is possible to use the repeat portion of the LytA molecule found in the C terminal end starting at residue 178, for example residues 188 - 305.

The present invention also includes variants of the aforementioned polypeptides, that is polypeptides that vary from the referents by conservative amino acid substitutions, whereby a residue is substituted by another with like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr.

Polypeptides of the present invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

It is most preferred that a polypeptide of the invention is derived from *Neisseria meningitidis*, however, it may preferably be obtained from other organisms of the same taxonomic genus. A polypeptide of the invention may also be obtained, for example, from organisms of the same taxonomic family or order.

Polynucleotides

It is an object of the invention to provide polynucleotides that encode BASB029 polypeptides, particularly polynucleotides that encode the polypeptide herein designated BASB029.

In a particularly preferred embodiment of the invention the polynucleotide comprises a region encoding BASB029 polypeptides comprising a sequence set out in SEQ ID NO:1,3 which includes a full length gene, or a variant thereof.

The BASB029 polynucleotides provided in SEQ ID NO:1,3 are the BASB029 polynucleotides from *Neisseria meningitidis* strains ATCC13090 and H44/76.

As a further aspect of the invention there are provided isolated nucleic acid molecules encoding and/or expressing BASB029 polypeptides and polynucleotides, particularly *Neisseria meningitidis* BASB029 polypeptides and polynucleotides, including, for example, unprocessed RNAs, ribozyme RNAs, mRNAs, cDNAs, genomic DNAs, B- and Z-DNAs. Further embodiments of the invention include biologically, diagnostically, prophylactically, clinically or therapeutically useful polynucleotides and polypeptides, and variants thereof, and compositions comprising the same.

Another aspect of the invention relates to isolated polynucleotides, including at least one full length gene, that encodes a BASB029 polypeptide having a deduced amino acid sequence of SEQ ID NO:2,4 and polynucleotides closely related thereto and variants thereof.

In another particularly preferred embodiment of the invention there is a BASB029 polypeptide from *Neisseria meningitidis* comprising or consisting of an amino acid sequence of SEQ ID NO:2,4 or a variant thereof.

Using the information provided herein, such as a polynucleotide sequence set out in SEQ ID NO:1, 3 a polynucleotide of the invention encoding BASB029 polypeptide may be obtained using standard cloning and screening methods, such as those for cloning and sequencing chromosomal DNA fragments from bacteria using *Neisseria meningitidis* cells as starting material, followed by obtaining a full length clone. For example, to obtain a polynucleotide sequence of the invention, such as a polynucleotide sequence given in SEQ ID NO:1,3,

typically a library of clones of chromosomal DNA of *Neisseria meningitidis* in *E.coli* or some other suitable host is probed with a radiolabeled oligonucleotide, preferably a 17-mer or longer, derived from a partial sequence. Clones carrying DNA identical to that of the probe can then be distinguished using stringent hybridization conditions. By sequencing the individual clones thus identified by hybridization with sequencing primers designed from the original polypeptide or polynucleotide sequence it is then possible to extend the polynucleotide sequence in both directions to determine a full length gene sequence. Conveniently, such sequencing is performed, for example, using denatured double stranded DNA prepared from a plasmid clone. Suitable techniques are described by Maniatis, T., Fritsch, E.F. and Sambrook et al., *MOLECULAR CLONING, A LABORATORY MANUAL*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989). (see in particular Screening By Hybridization 1.90 and Sequencing Denatured Double-Stranded DNA Templates 13.70). Direct genomic DNA sequencing may also be performed to obtain a full length gene sequence. Illustrative of the invention, each polynucleotide set out in SEQ ID NO:1,3 was discovered in a DNA library derived from *Neisseria meningitidis*.

Moreover, each DNA sequence set out in SEQ ID NO:1,3 contains an open reading frame encoding a protein having about the number of amino acid residues set forth in SEQ ID NO:2, 4 with a deduced molecular weight that can be calculated using amino acid residue molecular weight values well known to those skilled in the art.

The polynucleotide of SEQ ID NO:1, between the start codon at nucleotide number 1 and the stop codon which begins at nucleotide number 1783 of SEQ ID NO:1, encodes the polypeptide of SEQ ID NO:2.

The polynucleotide of SEQ ID NO:3, between the start codon at nucleotide number 1 and the stop codon which begins at nucleotide number 1774 of SEQ ID NO:3, encodes the polypeptide of SEQ ID NO:4.

In a further aspect, the present invention provides for an isolated polynucleotide comprising or consisting of:

(a) a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:1,3 over the entire length of SEQ ID NO:1,3 respectively; or

(b) a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or 100% exact, to the amino acid sequence of SEQ ID NO:2, 4 over the entire length of SEQ ID NO:2, 4 respectively.

A polynucleotide encoding a polypeptide of the present invention, including homologs and orthologs from species other than *Neisseria meningitidis*, may be obtained by a process which comprises the steps of screening an appropriate library under stringent hybridization conditions (for example, using a temperature in the range of 45 – 65°C and an SDS concentration from 0.1 – 1%) with a labeled or detectable probe consisting of or comprising the sequence of SEQ ID NO: 1, 3 or a fragment thereof; and isolating a full-length gene and/or genomic clones containing said polynucleotide sequence.

The invention provides a polynucleotide sequence identical over its entire length to a coding sequence (open reading frame) in SEQ ID NO: 1, 3. Also provided by the invention is a coding sequence for a mature polypeptide or a fragment thereof, by itself as well as a coding sequence for a mature polypeptide or a fragment in reading frame with another coding sequence, such as a sequence encoding a leader or secretory sequence, a pre-, or pro- or prepro-protein sequence. The polynucleotide of the invention may also contain at least one non-coding sequence, including for example, but not limited to at least one non-coding 5' and 3' sequence, such as the transcribed but non-translated sequences, termination signals (such as rho-dependent and rho-independent termination signals), ribosome binding sites, Kozak sequences, sequences that stabilize mRNA, introns, and polyadenylation signals.

The polynucleotide sequence may also comprise additional coding sequence encoding additional amino acids. For example, a marker sequence that facilitates purification of the fused polypeptide can be encoded. In certain embodiments of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz *et al.*, *Proc. Natl. Acad. Sci., USA 86:* 821-824 (1989), or an HA peptide tag (Wilson *et al.*, *Cell 37:* 767 (1984), both of which may be useful in purifying polypeptide sequence fused to them. Polynucleotides of the invention also include, but are not limited to, polynucleotides comprising a structural gene and its naturally associated sequences that control gene expression.

The nucleotide sequence encoding BASB029 polypeptide of SEQ ID NO:2, 4 may be identical to the polypeptide encoding sequence contained in nucleotides 1 to 1782 of SEQ ID NO:1, or the polypeptide encoding sequence contained in nucleotides 1 to 1773 of SEQ ID NO:3, respectively. Alternatively it may be a sequence, which as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:2, 4.

The term "polynucleotide encoding a polypeptide" as used herein encompasses polynucleotides that include a sequence encoding a polypeptide of the invention, particularly a bacterial polypeptide and more particularly a polypeptide of the *Neisseria meningitidis* BASB029 having an amino acid sequence set out in SEQ ID NO:2, 4. The term also encompasses polynucleotides that include a single continuous region or discontinuous regions encoding the polypeptide (for example, polynucleotides interrupted by integrated phage, an integrated insertion sequence, an integrated vector sequence, an integrated transposon sequence, or due to RNA editing or genomic DNA reorganization) together with additional regions, that also may contain coding and/or non-coding sequences.

The invention further relates to variants of the polynucleotides described herein that encode variants of a polypeptide having a deduced amino acid sequence of SEQ ID NO:2, 4.

Fragments of polynucleotides of the invention may be used, for example, to synthesize full-length polynucleotides of the invention.

Further particularly preferred embodiments are polynucleotides encoding BASB029 variants, that have the amino acid sequence of BASB029 polypeptide of SEQ ID NO:2, 4 in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, modified, deleted and/or added, in any combination. Especially preferred among these are silent substitutions, additions and deletions, that do not alter the properties and activities of BASB029 polypeptide.

Further preferred embodiments of the invention are polynucleotides that are at least 85% identical over their entire length to a polynucleotide encoding BASB029 polypeptide having an amino acid sequence set out in SEQ ID NO:2, 4, and polynucleotides that are complementary to such polynucleotides. In this regard, polynucleotides at least 90% identical over their entire length to the same are particularly preferred, and among these particularly preferred polynucleotides, those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred among those with at least 95%, and among these those with at least 98% and at least 99% are particularly highly preferred, with at least 99% being the more preferred.

Preferred embodiments are polynucleotides encoding polypeptides that retain substantially the same biological function or activity as the mature polypeptide encoded by a DNA of SEQ ID NO:1, 3.

In accordance with certain preferred embodiments of this invention there are provided polynucleotides that hybridize, particularly under stringent conditions, to BASB029 polynucleotide sequences, such as those polynucleotides in SEQ ID NO:1, 3.

The invention further relates to polynucleotides that hybridize to the polynucleotide sequences provided herein. In this regard, the invention especially relates to polynucleotides that hybridize under stringent conditions to the polynucleotides described herein. As herein used, the terms "stringent conditions" and "stringent hybridization conditions" mean hybridization occurring only if there is at least 95% and preferably at least 97% identity between the sequences. A specific example of stringent hybridization conditions is overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/ml of denatured, sheared salmon sperm DNA, followed by washing the hybridization support in 0.1x SSC at about 65°C. Hybridization and wash conditions are well known and exemplified in Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), particularly Chapter 11 therein. Solution hybridization may also be used with the polynucleotide sequences provided by the invention.

The invention also provides a polynucleotide consisting of or comprising a polynucleotide sequence obtained by screening an appropriate library containing the complete gene for a polynucleotide sequence set forth in SEQ ID NO:1, 3 under stringent hybridization conditions with a probe having the sequence of said polynucleotide sequence set forth in SEQ ID NO:1, 3 or a fragment thereof; and isolating said polynucleotide sequence. Fragments useful for obtaining such a polynucleotide include, for example, probes and primers fully described elsewhere herein.

As discussed elsewhere herein regarding polynucleotide assays of the invention, for instance, the polynucleotides of the invention, may be used as a hybridization probe for RNA, cDNA and genomic DNA to isolate full-length cDNAs and genomic clones encoding BASB029 and to isolate cDNA and genomic clones of other genes that have a high identity, particularly high sequence identity, to the BASB029 gene. Such probes generally will comprise at least 15 nucleotide residues or base pairs. Preferably, such probes will have at

least 30 nucleotide residues or base pairs and may have at least 50 nucleotide residues or base pairs. Particularly preferred probes will have at least 20 nucleotide residues or base pairs and will have less than 30 nucleotide residues or base pairs.

A coding region of a BASB029 gene may be isolated by screening using a DNA sequence provided in SEQ ID NO:1, 3 to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the invention is then used to screen a library of cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.

There are several methods available and well known to those skilled in the art to obtain full-length DNAs, or extend short DNAs, for example those based on the method of Rapid Amplification of cDNA ends (RACE) (see, for example, Frohman, *et al.*, *PNAS USA 85:* 8998-9002, 1988). Recent modifications of the technique, exemplified by the Marathon™ technology (Clontech Laboratories Inc.) for example, have significantly simplified the search for longer cDNAs. In the Marathon™ technology, cDNAs have been prepared from mRNA extracted from a chosen tissue and an 'adaptor' sequence ligated onto each end. Nucleic acid amplification (PCR) is then carried out to amplify the "missing" 5' end of the DNA using a combination of gene specific and adaptor specific oligonucleotide primers. The PCR reaction is then repeated using "nested" primers, that is, primers designed to anneal within the amplified product (typically an adaptor specific primer that anneals further 3' in the adaptor sequence and a gene specific primer that anneals further 5' in the selected gene sequence). The products of this reaction can then be analyzed by DNA sequencing and a full-length DNA constructed either by joining the product directly to the existing DNA to give a complete sequence, or carrying out a separate full-length PCR using the new sequence information for the design of the 5' primer.

The polynucleotides and polypeptides of the invention may be employed, for example, as research reagents and materials for discovery of treatments of and diagnostics for diseases, particularly human diseases, as further discussed herein relating to polynucleotide assays.

The polynucleotides of the invention that are oligonucleotides derived from a sequence of SEQ ID NOS:1 – 4 may be used in the processes herein as described, but preferably for PCR, to determine whether or not the polynucleotides identified herein in whole or in part are transcribed in bacteria in infected tissue. It is recognized that such sequences will also have utility in diagnosis of the stage of infection and type of infection the pathogen has attained.

The invention also provides polynucleotides that encode a polypeptide that is the mature protein plus additional amino or carboxyl-terminal amino acids, or amino acids interior to the mature polypeptide (when the mature form has more than one polypeptide chain, for instance). Such sequences may play a role in processing of a protein from precursor to a mature form, may allow protein transport, may lengthen or shorten protein half-life or may facilitate manipulation of a protein for assay or production, among other things. As generally is the case *in vivo*, the additional amino acids may be processed away from the mature protein by cellular enzymes.

For each and every polynucleotide of the invention there is provided a polynucleotide complementary to it. It is preferred that these complementary polynucleotides are fully complementary to each polynucleotide with which they are complementary.

A precursor protein, having a mature form of the polypeptide fused to one or more prosequences may be an inactive form of the polypeptide. When prosequences are removed such inactive precursors generally are activated. Some or all of the prosequences may be removed before activation. Generally, such precursors are called proproteins.

In addition to the standard A, G, C, T/U representations for nucleotides, the term "N" may also be used in describing certain polynucleotides of the invention. "N" means that any of the four DNA or RNA nucleotides may appear at such a designated position in the DNA or RNA sequence, except it is preferred that N is not a nucleic acid that when taken in combination with adjacent nucleotide positions, when read in the correct reading frame, would have the effect of generating a premature termination codon in such reading frame.

In sum, a polynucleotide of the invention may encode a mature protein, a mature protein plus a leader sequence (which may be referred to as a preprotein), a precursor of a mature protein having one or more prosequences that are not the leader sequences of a preprotein, or a preproprotein, which is a precursor to a proprotein, having a leader sequence and one or more prosequences, which generally are removed during processing steps that produce active and mature forms of the polypeptide.

In accordance with an aspect of the invention, there is provided the use of a polynucleotide of the invention for therapeutic or prophylactic purposes, in particular genetic immunization.

The use of a polynucleotide of the invention in genetic immunization will preferably employ a suitable delivery method such as direct injection of plasmid DNA into muscles (Wolff *et al.*, *Hum Mol Genet* (1992) 1: 363, Manthorpe *et al.*, *Hum. Gene Ther.* (1983) 4: 419), delivery of DNA complexed with specific protein carriers (Wu *et al.*, *J Biol Chem.* (1989) 264: 16985), coprecipitation of DNA with calcium phosphate (Benvenisty & Reshef, *PNAS USA*, (1986) 83: 9551), encapsulation of DNA in various forms of liposomes (Kaneda *et al.*, *Science* (1989) 243: 375), particle bombardment (Tang *et al.*, *Nature* (1992) 356:152, Eisenbraun *et al.*, *DNA Cell Biol* (1993) 12: 791) and *in vivo* infection using cloned retroviral vectors (Seeger *et al.*, *PNAS USA* (1984) 81: 5849).

Vectors, Host Cells, Expression Systems

The invention also relates to vectors that comprise a polynucleotide or polynucleotides of the invention, host cells that are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the invention.

Recombinant polypeptides of the present invention may be prepared by processes well known in those skilled in the art from genetically engineered host cells comprising expression systems. Accordingly, in a further aspect, the present invention relates to expression systems that comprise a polynucleotide or polynucleotides of the present invention, to host cells which are genetically engineered with such expression systems, and to the production of polypeptides of the invention by recombinant techniques.

For recombinant production of the polypeptides of the invention, host cells can be genetically engineered to incorporate expression systems or portions thereof or polynucleotides of the invention. Introduction of a polynucleotide into the host cell can be effected by methods described in many standard laboratory manuals, such as Davis, *et al.*, *BASIC METHODS IN MOLECULAR BIOLOGY*, (1986) and Sambrook, *et al.*, *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989), such as, calcium phosphate transfection, DEAE-dextran mediated transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction and infection.

Representative examples of appropriate hosts include bacterial cells, such as cells of streptococci, staphylococci, enterococci, *E. coli*, streptomyces, cyanobacteria, *Bacillus subtilis*, *Moraxella catarrhalis*, *Haemophilus influenzae* and *Neisseria meningitidis*; fungal cells, such as cells of a yeast, *Kluveromyces*, *Saccharomyces*, a basidiomycete, *Candida*

albicans and *Aspergillus*; insect cells such as cells of *Drosophila* S2 and *Spodoptera* Sf9; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, 293, CV-1 and Bowes melanoma cells; and plant cells, such as cells of a gymnosperm or angiosperm.

A great variety of expression systems can be used to produce the polypeptides of the invention. Such vectors include, among others, chromosomal-, episomal- and virus-derived vectors, for example, vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses, picornaviruses, retroviruses, and alphaviruses and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression system constructs may contain control regions that regulate as well as engender expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides and/or to express a polypeptide in a host may be used for expression in this regard. The appropriate DNA sequence may be inserted into the expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook *et al.*, *MOLECULAR CLONING, A LABORATORY MANUAL*, (*supra*).

In recombinant expression systems in eukaryotes, for secretion of a translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the expressed polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals.

Polypeptides of the present invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography,

hydroxylapatite chromatography and lectin chromatography. Most preferably, ion metal affinity chromatography (IMAC) is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during intracellular synthesis, isolation and or purification.

The expression system may also be a recombinant live microorganism, such as a virus or bacterium. The gene of interest can be inserted into the genome of a live recombinant virus or bacterium. Inoculation and *in vivo* infection with this live vector will lead to *in vivo* expression of the antigen and induction of immune responses. Viruses and bacteria used for this purpose are for instance: poxviruses (e.g; vaccinia, fowlpox, canarypox), alphaviruses (Sindbis virus, Semliki Forest Virus, Venezuelian Equine Encephalitis Virus), adenoviruses, adeno-associated virus, picornaviruses (poliovirus, rhinovirus), herpesviruses (varicella zoster virus, etc), Listeria, Salmonella , Shigella, Neisseria, BCG. These viruses and bacteria can be virulent, or attenuated in various ways in order to obtain live vaccines. Such live vaccines also form part of the invention.

Diagnostic, Prognostic, Serotyping and Mutation Assays

This invention is also related to the use of BASB029 polynucleotides and polypeptides of the invention for use as diagnostic reagents. Detection of BASB029 polynucleotides and/or polypeptides in a eukaryote, particularly a mammal, and especially a human, will provide a diagnostic method for diagnosis of disease, staging of disease or response of an infectious organism to drugs. Eukaryotes, particularly mammals, and especially humans, particularly those infected or suspected to be infected with an organism comprising the BASB029 gene or protein, may be detected at the nucleic acid or amino acid level by a variety of well known techniques as well as by methods provided herein.

Polypeptides and polynucleotides for prognosis, diagnosis or other analysis may be obtained from a putatively infected and/or infected individual's bodily materials. Polynucleotides from any of these sources, particularly DNA or RNA, may be used directly for detection or

may be amplified enzymatically by using PCR or any other amplification technique prior to analysis. RNA, particularly mRNA, cDNA and genomic DNA may also be used in the same ways. Using amplification, characterization of the species and strain of infectious or resident organism present in an individual, may be made by an analysis of the genotype of a selected polynucleotide of the organism. Deletions and insertions can be detected by a change in size of the amplified product in comparison to a genotype of a reference sequence selected from a related organism, preferably a different species of the same genus or a different strain of the same species. Point mutations can be identified by hybridizing amplified DNA to labeled BASB029 polynucleotide sequences. Perfectly or significantly matched sequences can be distinguished from imperfectly or more significantly mismatched duplexes by DNase or RNase digestion, for DNA or RNA respectively, or by detecting differences in melting temperatures or renaturation kinetics. Polynucleotide sequence differences may also be detected by alterations in the electrophoretic mobility of polynucleotide fragments in gels as compared to a reference sequence. This may be carried out with or without denaturing agents. Polynucleotide differences may also be detected by direct DNA or RNA sequencing. See, for example, Myers *et al.*, *Science, 230:* 1242 (1985). Sequence changes at specific locations also may be revealed by nuclease protection assays, such as RNase, V1 and S1 protection assay or a chemical cleavage method. See, for example, Cotton *et al.*, *Proc. Natl. Acad. Sci., USA; 85:* 4397-4401 (1985).

In another embodiment, an array of oligonucleotides probes comprising BASB029 nucleotide sequence or fragments thereof can be constructed to conduct efficient screening of, for example, genetic mutations, serotype, taxonomic classification or identification. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability (see, for example, Chee *et al.*, *Science, 274: 610* (1996)).

Thus in another aspect, the present invention relates to a diagnostic kit which comprises:

(a) a polynucleotide of the present invention, preferably the nucleotide sequence of SEQ ID NO:1, 3, or a fragment thereof ;

(b) a nucleotide sequence complementary to that of (a);

(c) a polypeptide of the present invention, preferably the polypeptide of SEQ ID NO:2, 4 or a fragment thereof; or

(d) an antibody to a polypeptide of the present invention, preferably to the polypeptide of SEQ ID NO:2, 4.

It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component. Such a kit will be of use in diagnosing a disease or susceptibility to a disease, among others.

This invention also relates to the use of polynucleotides of the present invention as diagnostic reagents. Detection of a mutated form of a polynucleotide of the invention, preferable, SEQ ID NO:1, 3, which is associated with a disease or pathogenicity will provide a diagnostic tool that can add to, or define, a diagnosis of a disease, a prognosis of a course of disease, a determination of a stage of disease, or a susceptibility to a disease, which results from under-expression, over-expression or altered expression of the polynucleotide. Organisms, particularly infectious organisms, carrying mutations in such polynucleotide may be detected at the polynucleotide level by a variety of techniques, such as those described elsewhere herein.

Cells from an organism carrying mutations or polymorphisms (allelic variations) in a polynucleotide and/or polypeptide of the invention may also be detected at the polynucleotide or polypeptide level by a variety of techniques, to allow for serotyping, for example. For example, RT-PCR can be used to detect mutations in the RNA. It is particularly preferred to use RT-PCR in conjunction with automated detection systems, such as, for example, GeneScan. RNA, cDNA or genomic DNA may also be used for the same

purpose, PCR. As an example, PCR primers complementary to a polynucleotide encoding BASB029 polypeptide can be used to identify and analyze mutations.

The invention further provides primers with 1, 2, 3 or 4 nucleotides removed from the 5' and/or the 3' end. These primers may be used for, among other things, amplifying BASB029 DNA and/or RNA isolated from a sample derived from an individual, such as a bodily material. The primers may be used to amplify a polynucleotide isolated from an infected individual, such that the polynucleotide may then be subject to various techniques for elucidation of the polynucleotide sequence. In this way, mutations in the polynucleotide sequence may be detected and used to diagnose and/or prognose the infection or its stage or course, or to serotype and/or classify the infectious agent.

The invention further provides a process for diagnosing disease, preferably bacterial infections, more preferably infections caused by *Neisseria meningitidis*, comprising determining from a sample derived from an individual, such as a bodily material, an increased level of expression of polynucleotide having a sequence of SEQ ID NO:1, 3. Increased or decreased expression of a BASB029 polynucleotide can be measured using any on of the methods well known in the art for the quantitation of polynucleotides, such as, for example, amplification, PCR, RT-PCR, RNase protection, Northern blotting, spectrometry and other hybridization methods.

In addition, a diagnostic assay in accordance with the invention for detecting over-expression of BASB029 polypeptide compared to normal control tissue samples may be used to detect the presence of an infection, for example. Assay techniques that can be used to determine levels of a BASB029 polypeptide, in a sample derived from a host, such as a bodily material, are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis, antibody sandwich assays, antibody detection and ELISA assays.

The polynucleotides of the invention may be used as components of polynucleotide arrays, preferably high density arrays or grids. These high density arrays are particularly useful for diagnostic and prognostic purposes. For example, a set of spots each comprising a different gene, and further comprising a polynucleotide or polynucleotides of the invention, may be used for probing, such as using hybridization or nucleic acid amplification, using a probe obtained or derived from a bodily sample, to determine the presence of a particular polynucleotide sequence or related sequence in an individual. Such a presence may indicate the presence of a pathogen, particularly *Neisseria meningitidis*, and may be useful in diagnosing and/or prognosing disease or a course of disease. A grid comprising a number of variants of the polynucleotide sequence of SEQ ID NO:1, 3 are preferred. Also preferred is a grid comprising a number of variants of a polynucleotide sequence encoding the polypeptide sequence of SEQ ID NO:2, 4.

Antibodies

The polypeptides and polynucleotides of the invention or variants thereof, or cells expressing the same can be used as immunogens to produce antibodies immunospecific for such polypeptides or polynucleotides respectively.

In certain preferred embodiments of the invention there are provided antibodies against BASB029 polypeptides or polynucleotides.

Antibodies generated against the polypeptides or polynucleotides of the invention can be obtained by administering the polypeptides and/or polynucleotides of the invention, or epitope-bearing fragments of either or both, analogues of either or both, or cells expressing either or both, to an animal, preferably a nonhuman, using routine protocols. For preparation of monoclonal antibodies, any technique known in the art that provides antibodies produced by continuous cell line cultures can be used. Examples include various techniques, such as those in Kohler, G. and Milstein, C., *Nature 256:* 495-497 (1975);

Kozbor *et al.*, *Immunology Today 4:* 72 (1983); Cole *et al.*, pg. 77-96 in *MONOCLONAL ANTIBODIES AND CANCER THERAPY*, Alan R. Liss, Inc. (1985).

Techniques for the production of single chain antibodies (U.S. Patent No. 4,946,778) can be adapted to produce single chain antibodies to polypeptides or polynucleotides of this invention. Also, transgenic mice, or other organisms or animals, such as other mammals, may be used to express humanized antibodies immunospecific to the polypeptides or polynucleotides of the invention.

Alternatively, phage display technology may be utilized to select antibody genes with binding activities towards a polypeptide of the invention either from repertoires of PCR amplified v-genes of lymphocytes from humans screened for possessing anti-BASB029 or from naive libraries (McCafferty, *et al.*, (1990), Nature 348, 552-554; Marks, *et al.*, (1992) *Biotechnology 10*, 779-783). The affinity of these antibodies can also be improved by, for example, chain shuffling (Clackson *et al.*, (1991) *Nature 352:* 628).

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptides or polynucleotides of the invention to purify the polypeptides or polynucleotides by, for example, affinity chromatography.

Thus, among others, antibodies against BASB029-polypeptide or BASB029-polynucleotide may be employed to treat infections, particularly bacterial infections.

Polypeptide variants include antigenically, epitopically or immunologically equivalent variants form a particular aspect of this invention.

Preferably, the antibody or variant thereof is modified to make it less immunogenic in the individual. For example, if the individual is human the antibody may most preferably be "humanized," where the complimentarity determining region or regions of the hybridoma-

derived antibody has been transplanted into a human monoclonal antibody, for example as described in Jones *et al.* (1986), *Nature* 321, 522-525 or Tempest *et al.*, (1991) *Biotechnology* 9, 266-273.

Antagonists and Agonists - Assays and Molecules

Polypeptides and polynucleotides of the invention may also be used to assess the binding of small molecule substrates and ligands in, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These substrates and ligands may be natural substrates and ligands or may be structural or functional mimetics. See, *e.g.*, Coligan *et al.*, *Current Protocols in Immunology 1(2):* Chapter 5 (1991).

The screening methods may simply measure the binding of a candidate compound to the polypeptide or polynucleotide, or to cells or membranes bearing the polypeptide or polynucleotide, or a fusion protein of the polypeptide by means of a label directly or indirectly associated with the candidate compound. Alternatively, the screening method may involve competition with a labeled competitor. Further, these screening methods may test whether the candidate compound results in a signal generated by activation or inhibition of the polypeptide or polynucleotide, using detection systems appropriate to the cells comprising the polypeptide or polynucleotide. Inhibitors of activation are generally assayed in the presence of a known agonist and the effect on activation by the agonist by the presence of the candidate compound is observed. Constitutively active polypeptide and/or constitutively expressed polypeptides and polynucleotides may be employed in screening methods for inverse agonists or inhibitors, in the absence of an agonist or inhibitor, by testing whether the candidate compound results in inhibition of activation of the polypeptide or polynucleotide, as the case may be. Further, the screening methods may simply comprise the steps of mixing a candidate compound with a solution containing a polypeptide or polynucleotide of the present invention, to form a mixture, measuring BASB029 polypeptide and/or polynucleotide activity in the mixture, and

comparing the BASB029 polypeptide and/or polynucleotide activity of the mixture to a standard. Fusion proteins, such as those made from Fc portion and BASB029 polypeptide, as hereinbefore described, can also be used for high-throughput screening assays to identify antagonists of the polypeptide of the present invention, as well as of phylogenetically and and/or functionally related polypeptides (see D. Bennett *et al.*, J Mol Recognition, 8:52-58 (1995); and K. Johanson *et al.*, J Biol Chem, 270(16):9459-9471 (1995)).

The polynucleotides, polypeptides and antibodies that bind to and/or interact with a polypeptide of the present invention may also be used to configure screening methods for detecting the effect of added compounds on the production of mRNA and/or polypeptide in cells. For example, an ELISA assay may be constructed for measuring secreted or cell associated levels of polypeptide using monoclonal and polyclonal antibodies by standard methods known in the art. This can be used to discover agents which may inhibit or enhance the production of polypeptide (also called antagonist or agonist, respectively) from suitably manipulated cells or tissues.

The invention also provides a method of screening compounds to identify those which enhance (agonist) or block (antagonist) the action of BASB029 polypeptides or polynucleotides, particularly those compounds that are bacteristatic and/or bactericidal. The method of screening may involve high-throughput techniques. For example, to screen for agonists or antagonists, a synthetic reaction mix, a cellular compartment, such as a membrane, cell envelope or cell wall, or a preparation of any thereof, comprising BASB029 polypeptide and a labeled substrate or ligand of such polypeptide is incubated in the absence or the presence of a candidate molecule that may be a BASB029 agonist or antagonist. The ability of the candidate molecule to agonize or antagonize the BASB029 polypeptide is reflected in decreased binding of the labeled ligand or decreased production of product from such substrate. Molecules that bind gratuitously, *i.e.*, without inducing the effects of BASB029 polypeptide are most likely to be good antagonists. Molecules that bind well

and, as the case may be, increase the rate of product production from substrate, increase signal transduction, or increase chemical channel activity are agonists. Detection of the rate or level of, as the case may be, production of product from substrate, signal transduction, or chemical channel activity may be enhanced by using a reporter system. Reporter systems that may be useful in this regard include but are not limited to colorimetric, labeled substrate converted into product, a reporter gene that is responsive to changes in BASB029 polynucleotide or polypeptide activity, and binding assays known in the art.

Another example of an assay for BASB029 agonists is a competitive assay that combines BASB029 and a potential agonist with BASB029-binding molecules, recombinant BASB029 binding molecules, natural substrates or ligands, or substrate or ligand mimetics, under appropriate conditions for a competitive inhibition assay. BASB029 can be labeled, such as by radioactivity or a colorimetric compound, such that the number of BASB029 molecules bound to a binding molecule or converted to product can be determined accurately to assess the effectiveness of the potential antagonist.

Potential antagonists include, among others, small organic molecules, peptides, polypeptides and antibodies that bind to a polynucleotide and/or polypeptide of the invention and thereby inhibit or extinguish its activity or expression. Potential antagonists also may be small organic molecules, a peptide, a polypeptide such as a closely related protein or antibody that binds the same sites on a binding molecule, such as a binding molecule, without inducing BASB029-induced activities, thereby preventing the action or expression of BASB029 polypeptides and/or polynucleotides by excluding BASB029 polypeptides and/or polynucleotides from binding.

Potential antagonists include a small molecule that binds to and occupies the binding site of the polypeptide thereby preventing binding to cellular binding molecules, such that normal biological activity is prevented. Examples of small molecules include but are not limited to small organic molecules, peptides or peptide-like molecules. Other potential antagonists

include antisense molecules (see Okano, *J. Neurochem. 56:* 560 (1991); *OLIGODEOXYNUCLEOTIDES AS ANTISENSE INHIBITORS OF GENE EXPRESSION*, CRC Press, Boca Raton, FL (1988), for a description of these molecules). Preferred potential antagonists include compounds related to and variants of BASB029.

In a further aspect, the present invention relates to genetically engineered soluble fusion proteins comprising a polypeptide of the present invention, or a fragment thereof, and various portions of the constant regions of heavy or light chains of immunoglobulins of various subclasses (IgG, IgM, IgA, IgE). Preferred as an immunoglobulin is the constant part of the heavy chain of human IgG, particularly IgG1, where fusion takes place at the hinge region. In a particular embodiment, the Fc part can be removed simply by incorporation of a cleavage sequence which can be cleaved with blood clotting factor Xa. Furthermore, this invention relates to processes for the preparation of these fusion proteins by genetic engineering, and to the use thereof for drug screening, diagnosis and therapy. A further aspect of the invention also relates to polynucleotides encoding such fusion proteins. Examples of fusion protein technology can be found in International Patent Application Nos. WO94/29458 and WO94/22914.

Each of the polynucleotide sequences provided herein may be used in the discovery and development of antibacterial compounds. The encoded protein, upon expression, can be used as a target for the screening of antibacterial drugs. Additionally, the polynucleotide sequences encoding the amino terminal regions of the encoded protein or Shine-Delgarno or other translation facilitating sequences of the respective mRNA can be used to construct antisense sequences to control the expression of the coding sequence of interest.

The invention also provides the use of the polypeptide, polynucleotide, agonist or antagonist of the invention to interfere with the initial physical interaction between a pathogen or pathogens and a eukaryotic, preferably mammalian, host responsible for sequelae of infection. In particular, the molecules of the invention may be used: in the

prevention of adhesion of bacteria, in particular gram positive and/or gram negative bacteria, to eukaryotic, preferably mammalian, extracellular matrix proteins on in-dwelling devices or to extracellular matrix proteins in wounds; to block bacterial adhesion between eukaryotic, preferably mammalian, extracellular matrix proteins and bacterial BASB029 proteins that mediate tissue damage and/or; to block the normal progression of pathogenesis in infections initiated other than by the implantation of in-dwelling devices or by other surgical techniques.

In accordance with yet another aspect of the invention, there are provided BASB029 agonists and antagonists, preferably bacteristatic or bactericidal agonists and antagonists.

The antagonists and agonists of the invention may be employed, for instance, to prevent, inhibit and/or treat diseases.

In a further aspect, the present invention relates to mimotopes of the polypeptide of the invention. A mimotope is a peptide sequence, sufficiently similar to the native peptide (sequentially or structurally), which is capable of being recognised by antibodies which recognise the native peptide; or is capable of raising antibodies which recognise the native peptide when coupled to a suitable carrier.

Peptide mimotopes may be designed for a particular purpose by addition, deletion or substitution of elected amino acids. Thus, the peptides may be modified for the purposes of ease of conjugation to a protein carrier. For example, it may be desirable for some chemical conjugation methods to include a terminal cysteine. In addition it may be desirable for peptides conjugated to a protein carrier to include a hydrophobic terminus distal from the conjugated terminus of the peptide, such that the free unconjugated end of the peptide remains associated with the surface of the carrier protein. Thereby presenting the peptide in a conformation which most closely resembles that of the peptide as found in the context of the whole native molecule. For example, the peptides

may be altered to have an N-terminal cysteine and a C-terminal hydrophobic amidated tail. Alternatively, the addition or substitution of a D-stereoisomer form of one or more of the amino acids may be performed to create a beneficial derivative, for example to enhance stability of the peptide.

Alternatively, peptide mimotopes may be identified using antibodies which are capable themselves of binding to the polypeptides of the present invention using techniques such as phage display technology (EP 0 552 267 B1). This technique, generates a large number of peptide sequences which mimic the structure of the native peptides and are, therefore, capable of binding to anti-native peptide antibodies, but may not necessarily themselves share significant sequence homology to the native polypeptide.

Vaccines

Another aspect of the invention relates to a method for inducing an immunological response in an individual, particularly a mammal, preferably humans, which comprises inoculating the individual with BASB029 polynucleotide and/or polypeptide, or a fragment or variant thereof, adequate to produce antibody and/ or T cell immune response to protect said individual from infection, particularly bacterial infection and most particularly *Neisseria meningitidis* infection. Also provided are methods whereby such immunological response slows bacterial replication. Yet another aspect of the invention relates to a method of inducing immunological response in an individual which comprises delivering to such individual a nucleic acid vector, sequence or ribozyme to direct expression of BASB029 polynucleotide and/or polypeptide, or a fragment or a variant thereof, for expressing BASB029 polynucleotide and/or polypeptide, or a fragment or a variant thereof *in vivo* in order to induce an immunological response, such as, to produce antibody and/ or T cell immune response, including, for example, cytokine-producing T cells or cytotoxic T cells, to protect said individual, preferably a human, from disease, whether that disease is already established within the individual or not. One example of administering the gene is by accelerating it into the desired cells as a coating on particles

or otherwise. Such nucleic acid vector may comprise DNA, RNA, a ribozyme, a modified nucleic acid, a DNA/RNA hybrid, a DNA-protein complex or an RNA-protein complex.

A further aspect of the invention relates to an immunological composition that when introduced into an individual, preferably a human, capable of having induced within it an immunological response, induces an immunological response in such individual to a BASB029 polynucleotide and/or polypeptide encoded therefrom, wherein the composition comprises a recombinant BASB029 polynucleotide and/or polypeptide encoded therefrom and/or comprises DNA and/or RNA which encodes and expresses an antigen of said BASB029 polynucleotide, polypeptide encoded therefrom, or other polypeptide of the invention. The immunological response may be used therapeutically or prophylactically and may take the form of antibody immunity and/or cellular immunity, such as cellular immunity arising from CTL or CD4+ T cells.

A BASB029 polypeptide or a fragment thereof may be fused with co-protein or chemical moiety which may or may not by itself produce antibodies, but which is capable of stabilizing the first protein and producing a fused or modified protein which will have antigenic and/or immunogenic properties, and preferably protective properties. Thus fused recombinant protein, preferably further comprises an antigenic co-protein, such as lipoprotein D from *Haemophilus influenzae*, Glutathione-S-transferase (GST) or beta-galactosidase, or any other relatively large co-protein which solubilizes the protein and facilitates production and purification thereof. Moreover, the co-protein may act as an adjuvant in the sense of providing a generalized stimulation of the immune system of the organism receiving the protein. The co-protein may be attached to either the amino- or carboxy-terminus of the first protein.

Provided by this invention are compositions, particularly vaccine compositions, and methods comprising the polypeptides and/or polynucleotides of the invention and

immunostimulatory DNA sequences, such as those described in Sato, Y. *et al.* Science 273: 352 (1996).

Also, provided by this invention are methods using the described polynucleotide or particular fragments thereof, which have been shown to encode non-variable regions of bacterial cell surface proteins, in polynucleotide constructs used in such genetic immunization experiments in animal models of infection with *Neisseria meningitidis*. Such experiments will be particularly useful for identifying protein epitopes able to provoke a prophylactic or therapeutic immune response. It is believed that this approach will allow for the subsequent preparation of monoclonal antibodies of particular value, derived from the requisite organ of the animal successfully resisting or clearing infection, for the development of prophylactic agents or therapeutic treatments of bacterial infection, particularly *Neisseria meningitidis* infection, in mammals, particularly humans.

The invention also includes a vaccine formulation which comprises an immunogenic recombinant polypeptide and/or polynucleotide of the invention together with a suitable carrier, such as a pharmaceutically acceptable carrier. Since the polypeptides and polynucleotides may be broken down in the stomach, each is preferably administered parenterally, including, for example, administration that is subcutaneous, intramuscular, intravenous, or intradermal. Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteristatic compounds and solutes which render the formulation isotonic with the bodily fluid, preferably the blood, of the individual; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use.

The vaccine formulation of the invention may also include adjuvant systems for enhancing the immunogenicity of the formulation. Preferably the adjuvant system raises preferentially a TH1 type of response.

An immune response may be broadly distinguished into two extreme catagories, being a humoral or cell mediated immune responses (traditionally characterised by antibody and cellular effector mechanisms of protection respectively). These categories of response have been termed TH1-type responses (cell-mediated response), and TH2-type immune responses (humoral response).

Extreme TH1-type immune responses may be characterised by the generation of antigen specific, haplotype restricted cytotoxic T lymphocytes, and natural killer cell responses. In mice TH1-type responses are often characterised by the generation of antibodies of the IgG2a subtype, whilst in the human these correspond to IgG1 type antibodies. TH2-type immune responses are characterised by the generation of a broad range of immunoglobulin isotypes including in mice IgG1, IgA, and IgM.

It can be considered that the driving force behind the development of these two types of immune responses are cytokines. High levels of TH1-type cytokines tend to favour the induction of cell mediated immune responses to the given antigen, whilst high levels of TH2-type cytokines tend to favour the induction of humoral immune responses to the antigen.

The distinction of TH1 and TH2-type immune responses is not absolute. In reality an individual will support an immune response which is described as being predominantly TH1 or predominantly TH2. However, it is often convenient to consider the families of cytokines in terms of that described in murine CD4 +ve T cell clones by Mosmann and Coffman (*Mosmann, T.R. and Coffman, R.L. (1989) TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annual Review*

of Immunology, 7, p145-173). Traditionally, TH1-type responses are associated with the production of the INF-γ and IL-2 cytokines by T-lymphocytes. Other cytokines often directly associated with the induction of TH1-type immune responses are not produced by T-cells, such as IL-12. In contrast, TH2- type responses are associated with the secretion of IL-4, IL-5, IL-6 and IL-13.

It is known that certain vaccine adjuvants are particularly suited to the stimulation of either TH1 or TH2 - type cytokine responses. Traditionally the best indicators of the TH1:TH2 balance of the immune response after a vaccination or infection includes direct measurement of the production of TH1 or TH2 cytokines by T lymphocytes *in vitro* after restimulation with antigen, and/or the measurement of the IgG1:IgG2a ratio of antigen specific antibody responses.

Thus, a TH1-type adjuvant is one which preferentially stimulates isolated T-cell populations to produce high levels of TH1-type cytokines when re-stimulated with antigen *in vitro*, and promotes development of both CD8+ cytotoxic T lymphocytes and antigen specific immunoglobulin responses associated with TH1-type isotype.

Adjuvants which are capable of preferential stimulation of the TH1 cell response are described in International Patent Application No. WO 94/00153 and WO 95/17209.

3 De-O-acylated monophosphoryl lipid A (3D-MPL) is one such adjuvant. This is known from GB 2220211 (Ribi). Chemically it is a mixture of 3 De-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains and is manufactured by Ribi Immunochem, Montana. A preferred form of 3 De-O-acylated monophosphoryl lipid A is disclosed in European Patent 0 689 454 B1 (SmithKline Beecham Biologicals SA).

Preferably, the particles of 3D-MPL are small enough to be sterile filtered through a 0.22micron membrane (European Patent number 0 689 454).

3D-MPL will be present in the range of 10μg - 100μg preferably 25-50μg per dose wherein the antigen will typically be present in a range 2-50μg per dose.

Another preferred adjuvant comprises QS21, an Hplc purified non-toxic fraction derived from the bark of Quillaja Saponaria Molina. Optionally this may be admixed with 3 De-O-acylated monophosphoryl lipid A (3D-MPL), optionally together with a carrier.

The method of production of QS21 is disclosed in US patent No. 5,057,540.

Non-reactogenic adjuvant formulations containing QS21 have been described previously (WO 96/33739). Such formulations comprising QS21 and cholesterol have been shown to be successful TH1 stimulating adjuvants when formulated together with an antigen.

Further adjuvants which are preferential stimulators of TH1 cell response include immunomodulatory oligonucleotides, for example unmethylated CpG sequences as disclosed in WO 96/02555.

Combinations of different TH1 stimulating adjuvants, such as those mentioned hereinabove, are also contemplated as providing an adjuvant which is a preferential stimulator of TH1 cell response. For example, QS21 can be formulated together with 3D-MPL. The ratio of QS21 : 3D-MPL will typically be in the order of 1 : 10 to 10 : 1; preferably 1:5 to 5 : 1 and often substantially 1 : 1. The preferred range for optimal synergy is 2.5 : 1 to 1 : 1 3D-MPL: QS21.

Preferably a carrier is also present in the vaccine composition according to the invention. The carrier may be an oil in water emulsion, or an aluminium salt, such as aluminium phosphate or aluminium hydroxide.

A preferred oil-in-water emulsion comprises a metabolisible oil, such as squalene, alpha tocopherol and Tween 80. In a particularly preferred aspect the antigens in the vaccine composition according to the invention are combined with QS21 and 3D-MPL in such an emulsion. Additionally the oil in water emulsion may contain span 85 and/or lecithin and/or tricaprylin.

Typically for human administration QS21 and 3D-MPL will be present in a vaccine in the range of 1μg - 200μg, such as 10-100μg, preferably 10μg - 50μg per dose. Typically the oil in water will comprise from 2 to 10% squalene, from 2 to 10% alpha tocopherol and from 0.3 to 3% tween 80. Preferably the ratio of squalene: alpha tocopherol is equal to or less than 1 as this provides a more stable emulsion. Span 85 may also be present at a level of 1%. In some cases it may be advantageous that the vaccines of the present invention will further contain a stabiliser.

Non-toxic oil in water emulsions preferably contain a non-toxic oil, e.g. squalane or squalene, an emulsifier, e.g. Tween 80, in an aqueous carrier. The aqueous carrier may be, for example, phosphate buffered saline.

A particularly potent adjuvant formulation involving QS21, 3D-MPL and tocopherol in an oil in water emulsion is described in WO 95/17210.

The present invention also provides a polyvalent vaccine composition comprising a vaccine formulation of the invention in combination with other antigens, in particular antigens useful for treating cancers, autoimmune diseases and related conditions. Such a polyvalent vaccine composition may include a TH-1 inducing adjuvant as hereinbefore described.

While the invention has been described with reference to certain BASB029 polypeptides and polynucleotides, it is to be understood that this covers fragments of the naturally

occurring polypeptides and polynucleotides, and similar polypeptides and polynucleotides with additions, deletions or substitutions which do not substantially affect the immunogenic properties of the recombinant polypeptides or polynucleotides.

The antigen can also be delivered in the form of whole bacteria (dead or alive) or as subcellular fractions, these possibilities do include *N.meningitidis* itself.

Compositions, kits and administration

In a further aspect of the invention there are provided compositions comprising a BASB029 polynucleotide and/or a BASB029 polypeptide for administration to a cell or to a multicellular organism.

The invention also relates to compositions comprising a polynucleotide and/or a polypeptide discussed herein or their agonists or antagonists. The polypeptides and polynucleotides of the invention may be employed in combination with a non-sterile or sterile carrier or carriers for use with cells, tissues or organisms, such as a pharmaceutical carrier suitable for administration to an individual. Such compositions comprise, for instance, a media additive or a therapeutically effective amount of a polypeptide and/or polynucleotide of the invention and a pharmaceutically acceptable carrier or excipient. Such carriers may include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol and combinations thereof. The formulation should suit the mode of administration. The invention further relates to diagnostic and pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

Polypeptides, polynucleotides and other compounds of the invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

The pharmaceutical compositions may be administered in any effective, convenient manner including, for instance, administration by topical, oral, anal, vaginal, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes among others.

In therapy or as a prophylactic, the active agent may be administered to an individual as an injectable composition, for example as a sterile aqueous dispersion, preferably isotonic.

In a further aspect, the present invention provides for pharmaceutical compositions comprising a therapeutically effective amount of a polypeptide and/or polynucleotide, such as the soluble form of a polypeptide and/or polynucleotide of the present invention, agonist or antagonist peptide or small molecule compound, in combination with a pharmaceutically acceptable carrier or excipient. Such carriers include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention. Polypeptides, polynucleotides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

The composition will be adapted to the route of administration, for instance by a systemic or an oral route. Preferred forms of systemic administration include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can be used. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if a polypeptide or other compounds of the present invention can be formulated in an enteric or an encapsulated formulation, oral administration may also be possible. Administration of these compounds may also be topical and/or localized, in the form of salves, pastes, gels, solutions, powders and the like.

For administration to mammals, and particularly humans, it is expected that the daily dosage level of the active agent will be from 0.01 mg/kg to 10 mg/kg, typically around 1 mg/kg. The physician in any event will determine the actual dosage which will be most suitable for an individual and will vary with the age, weight and response of the particular individual. The above dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

The dosage range required depends on the choice of peptide, the route of administration, the nature of the formulation, the nature of the subject's condition, and the judgment of the attending practitioner. Suitable dosages, however, are in the range of 0.1-100 μg/kg of subject.

A vaccine composition is conveniently in injectable form. Conventional adjuvants may be employed to enhance the immune response. A suitable unit dose for vaccination is 0.5-5 microgram/kg of antigen, and such dose is preferably administered 1-3 times and with an interval of 1-3 weeks. With the indicated dose range, no adverse toxicological effects will be observed with the compounds of the invention which would preclude their administration to suitable individuals.

Wide variations in the needed dosage, however, are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art.

Sequence Databases, Sequences in a Tangible Medium, and Algorithms

Polynucleotide and polypeptide sequences form a valuable information resource with which to determine their 2- and 3-dimensional structures as well as to identify further sequences of similar homology. These approaches are most easily facilitated by storing the sequence in a computer readable medium and then using the stored data in a known macromolecular structure program or to search a sequence database using well known searching tools, such as the GCG program package.

Also provided by the invention are methods for the analysis of character sequences or strings, particularly genetic sequences or encoded protein sequences. Preferred methods of sequence analysis include, for example, methods of sequence homology analysis, such as identity and similarity analysis, DNA, RNA and protein structure analysis, sequence assembly, cladistic analysis, sequence motif analysis, open reading frame determination, nucleic acid base calling, codon usage analysis, nucleic acid base trimming, and sequencing chromatogram peak analysis.

A computer based method is provided for performing homology identification. This method comprises the steps of: providing a first polynucleotide sequence comprising the sequence of a polynucleotide of the invention in a computer readable medium; and comparing said first polynucleotide sequence to at least one second polynucleotide or polypeptide sequence to identify homology.

A computer based method is also provided for performing homology identification, said method comprising the steps of: providing a first polypeptide sequence comprising the sequence of a polypeptide of the invention in a computer readable medium; and comparing said first polypeptide sequence to at least one second polynucleotide or polypeptide sequence to identify homology.

All publications and references, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference in their

entirety as if each individual publication or reference were specifically and individually indicated to be incorporated by reference herein as being fully set forth. Any patent application to which this application claims priority is also incorporated by reference herein in its entirety in the manner described above for publications and references.

DEFINITIONS

"Identity," as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as the case may be, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. "Identity" can be readily calculated by known methods, including but not limited to those described in (*Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data*, Part I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heine, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., SIAM *J. Applied Math., 48:* 1073 (1988). Methods to determine identity are designed to give the largest match between the sequences tested. Moreover, methods to determine identity are codified in publicly available computer programs. Computer program methods to determine identity between two sequences include, but are not limited to, the GAP program in the GCG program package (Devereux, J., et al., *Nucleic Acids Research 12(1):* 387 (1984)), BLASTP, BLASTN (Altschul, S.F. et al., *J. Molec. Biol. 215:* 403-410 (1990), and FASTA(Pearson and Lipman Proc. Natl. Acad. Sci. USA 85; 2444-2448 (1988). The BLAST family of programs is publicly available from NCBI and other sources (*BLAST Manual*, Altschul, S., *et al.*, NCBI NLM NIH Bethesda, MD 20894;

Altschul, S., *et al.*, *J. Mol. Biol. 215*: 403-410 (1990). The well known Smith Waterman algorithm may also be used to determine identity.

Parameters for polypeptide sequence comparison include the following:

Algorithm: Needleman and Wunsch, J. Mol Biol. 48: 443-453 (1970)

Comparison matrix: BLOSSUM62 from Henikoff and Henikoff,

Proc. Natl. Acad. Sci. USA. 89:10915-10919 (1992)

Gap Penalty: 8

Gap Length Penalty: 2

A program useful with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison WI. The aforementioned parameters are the default parameters for peptide comparisons (along with no penalty for end gaps).

Parameters for polynucleotide comparison include the following:

Algorithm: Needleman and Wunsch, J. Mol Biol. 48: 443-453 (1970)

Comparison matrix: matches = +10, mismatch = 0

Gap Penalty: 50

Gap Length Penalty: 3

Available as: The "gap" program from Genetics Computer Group, Madison WI. These are the default parameters for nucleic acid comparisons.

A preferred meaning for "identity" for polynucleotides and polypeptides, as the case may be, are provided in (1) and (2) below.

(1) Polynucleotide embodiments further include an isolated polynucleotide comprising a polynucleotide sequence having at least a 50, 60, 70, 80, 85, 90, 95, 97 or 100% identity to the reference sequence of SEQ ID NO:1, wherein said polynucleotide sequence may be identical to the reference sequence of SEQ ID NO:1 or may include up to a certain integer number of nucleotide alterations as compared to the reference

sequence, wherein said alterations are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence, and wherein said number of nucleotide alterations is determined by multiplying the total number of nucleotides in SEQ ID NO:1 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of nucleotides in SEQ ID NO:1, or:

$$n_n \leq x_n - (x_n \bullet y),$$

wherein n_n is the number of nucleotide alterations, x_n is the total number of nucleotides in SEQ ID NO:1, y is 0.50 for 50%, 0.60 for 60%, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and • is the symbol for the multiplication operator, and wherein any non-integer product of x_n and y is rounded down to the nearest integer prior to subtracting it from x_n. Alterations of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:2 may create nonsense, missense or frameshift mutations in this coding sequence and thereby alter the polypeptide encoded by the polynucleotide following such alterations.

By way of example, a polynucleotide sequence of the present invention may be identical to the reference sequence of SEQ ID NO:1, that is it may be 100% identical, or it may include up to a certain integer number of nucleic acid alterations as compared to the reference sequence such that the percent identity is less than 100% identity. Such alterations are selected from the group consisting of at least one nucleic acid deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5' or 3' terminal positions of the reference polynucleotide sequence or anywhere between those terminal positions, interspersed either individually

among the nucleic acids in the reference sequence or in one or more contiguous groups within the reference sequence. The number of nucleic acid alterations for a given percent identity is determined by multiplying the total number of nucleic acids in SEQ ID NO:1 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of nucleic acids in SEQ ID NO:1, or:

$$n_n \leq x_n - (x_n \bullet y),$$

wherein n_n is the number of nucleic acid alterations, x_n is the total number of nucleic acids in SEQ ID NO:1, y is, for instance 0.70 for 70%, 0.80 for 80%, 0.85 for 85% etc., • is the symbol for the multiplication operator, and wherein any non-integer product of x_n and y is rounded down to the nearest integer prior to subtracting it from x_n.

(2) Polypeptide embodiments further include an isolated polypeptide comprising a polypeptide having at least a 50, 60, 70, 80, 85, 90, 95, 97 or 100% identity to a polypeptide reference sequence of SEQ ID NO:2, wherein said polypeptide sequence may be identical to the reference sequence of SEQ ID NO:2 or may include up to a certain integer number of amino acid alterations as compared to the reference sequence, wherein said alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence, and wherein said number of amino acid alterations is determined by multiplying the total number of amino acids in SEQ ID NO:2 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of amino acids in SEQ ID NO:2, or:

$$n_a \leq x_a - (x_a \bullet y),$$

wherein n_a is the number of amino acid alterations, x_a is the total number of amino acids in SEQ ID NO:2, y is 0.50 for 50%, 0.60 for 60%, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and • is the symbol for the multiplication operator, and wherein any non-integer product of x_a and y is rounded down to the nearest integer prior to subtracting it from x_a.

By way of example, a polypeptide sequence of the present invention may be identical to the reference sequence of SEQ ID NO:2, that is it may be 100% identical, or it may include up to a certain integer number of amino acid alterations as compared to the reference sequence such that the percent identity is less than 100% identity. Such alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence. The number of amino acid alterations for a given % identity is determined by multiplying the total number of amino acids in SEQ ID NO:2 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of amino acids in SEQ ID NO:2, or:

$$n_a \leq x_a - (x_a \bullet y),$$

wherein n_a is the number of amino acid alterations, x_a is the total number of amino acids in SEQ ID NO:2, y is, for instance 0.70 for 70%, 0.80 for 80%, 0.85 for 85% etc., and • is the symbol for the multiplication operator, and wherein any non-integer product of x_a and y is rounded down to the nearest integer prior to subtracting it from x_a.

"Individual(s)," when used herein with reference to an organism, means a multicellular eukaryote, including, but not limited to a metazoan, a mammal, an ovid, a bovid, a simian, a primate, and a human.

"Isolated" means altered "by the hand of man" from its natural state, *i.e.*, if it occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living organism is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein. Moreover, a polynucleotide or polypeptide that is introduced into an organism by transformation, genetic manipulation or by any other recombinant method is "isolated" even if it is still present in said organism, which organism may be living or non-living.

"Polynucleotide(s)" generally refers to any polyribonucleotide or polydeoxyribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA including single and double-stranded regions.

"Variant" refers to a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino

acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

"Disease(s)" means any disease caused by or related to infection by a bacteria, including , for example, upper respiratory tract infection, invasive bacterial diseases, such as bacteremia and meningitis.

EXAMPLES:

The examples below are carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. The examples are illustrative, but do not limit the invention.

Example1: Discovery and confirmatory DNA sequencing of the BASB029 gene from two *N.meningitidis* strains.

A: BASB029 in *N. meningitidis* serogroup B strain ATCC13090.

The BASB029 gene of SEQ ID NO:1 was first discovered in the Incyte PathoSeq database containing unfinished genomic DNA sequences of the *N. meningitidis* strain ATCC13090. The translation of the BASB029 polynucleotide sequence, showed in SEQ ID NO:2, showed significant similarity (52 % identity in a 582 amino acids overlap) to the *Haemophilus influenzae* surface fibril (HSF) protein.

The sequence of the BASB029 gene was further confirmed experimentally. For this purpose, genomic DNA was extracted from 10^{10} cells of the *N.meningitidis* cells (strain ATCC 13090) using the QIAGEN genomic DNA extraction kit (Qiagen Gmbh), and 1µg of this material was submitted to Polymerase Chain Reaction DNA amplification using primers Hsf1 (5'- GGG GCA TAT GAA CAA AAT ATA CCG CAT CAT TTG GAA-3') [SEQ ID NO:5] containing an internal *Nde*I site (underlined) and Hsf2 (5'- GGG GCT CGA GCC ACT GAT AAC CGA CAG ATG CGG A-3') [SEQ ID NO:6] containing an internal *Xho*I site (underlined). This PCR product was gel-purified and subjected to DNA sequencing using the Big Dye Cycle Sequencing kit (Perkin-Elmer) and an ABI 373A/PRISM DNA sequencer. DNA sequencing was performed on both strands with a redundancy of 2 and the full-length sequence was assembled using the SeqMan program from the DNASTAR Lasergene software package The resulting DNA sequence turned out to be 100 % identical to SEQ ID NO:1.

B: BASB029 in *N. meningitidis* serogroup B strain H44/76.

The sequence of the BASB029 gene was also determined in another *N. meningitidis* serogroup B strain, the strain H44/76. For this purpose, genomic DNA was extracted from the *N. meningitidis* strain H44/76 using the experimental conditions presented in Example 1. This material (1µg) was then submitted to Polymerase Chain Reaction DNA amplification using primers Hsf1 and Hsf2 specific for the BASB029 gene. A 4389bp DNA fragment was obtained, digested by the *NdeI/XhoI* restriction endonucleases and inserted into the corresponding sites of the pET-24b cloning/expression vector (Novagen) using standard molecular biology techniques (Molecular Cloning,a Laboratory Manual, Second Edition, Eds: Sambrook, Fritsch & Maniatis, Cold Spring Harbor press 1989). Recombinant pET-24b/BASB029 was then submitted to DNA sequencing using the Big Dyes kit (Applied biosystems) and analyzed on a ABI 373/A DNA sequencer in the conditions described by the supplier. As a result, the polynucleotide and deduced polypeptide sequences, referred to as SEQ ID NO:3 and SEQ ID NO:4 respectively, were obtained. Using the PILEUP program from the GCG package, an alignment of the polynucleotide sequences of SEQ ID NO:1 and 3 was performed, and is displayed in Figure 1; their level of identity amounts to 96.8 % as determined by the GAP program. Using the same PILEUP program, an alignment of the polypeptide sequences of SEQ ID NO:2 and 4 was performed, and is displayed in Figure 2; their level of identity amounts to 94.2 %, as determined by the GAP program. Taken together, these data indicate strong sequence conservation of the BASB029 gene among the two *N.meningitidis* serogroup B strains.

Example 2: Expression and purification of recombinant BASB029 protein in *Escherichia coli.*

The construction of the pET-24b/BASB029 cloning/expression vector was described in Example 1B. This vector harbours the BASB029 gene isolated from the strain H44/76 in fusion with a stretch of 6 Histidine residues, placed under the control of the strong bacteriophage T7 gene 10 promoter. For expression study, this vector was introduced into the *Escherichia coli* strain Novablue (DE3) (Novagen), in which, the gene for the T7 polymerase is placed under the control of the isopropyl-beta-D thiogalactoside (IPTG)-regulatable *lac* promoter. Liquid cultures (100 ml) of the Novablue (DE3) [pET-24b/BASB029] *E. coli* recombinant strain were grown at 37°C under agitation until the optical density at 600nm (OD600) reached 0.6. At that time-point, IPTG was added at a final concentration of 1mM and the culture was grown for 4 additional hours. The culture was then centrifuged at 10,000 rpm and the pellet was frozen at -20°C for at least 10 hours. After thawing, the pellet was resuspended during 30 min at 25°C in buffer A (6M guanidine hydrochloride, 0.1M NaH2PO4, 0.01M Tris, pH 8.0), passed three-times through a needle and clarified by centrifugation (20000rpm, 15 min). The sample was then loaded at a flow-rate of 1ml/min on a Ni2+ -loaded Hitrap column (Pharmacia Biotech). After passsage of the flowthrough, the column was washed succesively with 40ml of buffer B (8M Urea, 0.1MNaH2PO4, 0.01M Tris, pH 8.0), 40ml of buffer C (8M Urea, 0.1MNaH2PO4, 0.01M Tris, pH 6.3). The recombinant protein BASB029/His6 was then eluted from the column with 30ml of buffer D (8M Urea, 0.1MNaH2PO4, 0.01M Tris, pH 6.3) containing 500mM of imidazole and 3ml-size fractions were collected. As shown in Figure 3 (lane1), a highly enriched (Purity estimated to more than 90% pure in coomassie staining) BASB029/His6 protein, migrating at 66kDa (estimated relative molecular mass), was eluted from the column. This polypeptide was reactive against a mouse monoclonal antibody raised against the 5-histidine motif (see figure 3, lane2). Taken together, these data indicate that the BASB029 gene can be expressed and purified under a recombinant form (BASB029/His6) in *E.coli*.

Example 3 : Immunization of mice with recombinant BASB029

Partially purified recombinant BASB029 protein expressed in E. coli has been injected three times in Balb/C mice on days 0, 14 and 29 (8 animals/group). Animals were injected by the subcutaneous route with around 5µg of antigen in two different formulations : either adsorbed on 100µg $AlPO_4$ or formulated in SBAS2 emulsion (SB62 emulsion containing 5µg MPL and 5µg QS21 per dose). A negative control group consisting of mice immunized with the SBAS2 emulsion only has also been added in the experiment. Mice were bled on days 29 (15 days Post II) and 35 (6 days Post III) in order to detect specific anti-BASB029 antibodies. Specific anti-BASB029 antibodies were measured on pooled sera (from 10 mice/group) by western-blotting on six different NmB strains (Figures 4 and 5).

Recognition of the BASB029 epitopes on different NmB strains by western-blotting

In this test, immunized mice sera (pooled) have been tested by western-blotting for recognition of the BASB029 epitopes on six different neisseria meningitidis B strains : H44/76 (B:15:P1.7, 16, lineage ET-5), M97 250987 (B:4:P1.15), BZ10 (B:2b:P1.2, lineage A4), BZ198 (B:NT*: -, lineage 3), EG328 (B:NT*, lineage ST-18), and the ATCC 13090 Men B strain, as well as on partially purified recombinant BASB029 protein (*** : NT : Not Typed).**

Briefly, 10µl (> 10^8 cells/lane) of each sample treated with sample buffer (10 min at 95°C) are put into a SDS-PAGE gradient gel (Tris-glycine 4-20%, Novex, code n°EC60252). Electrophoretic migration occurs at 125 volts for 90 min. Afterwards, proteins are transferred to nitrocellulose sheet (0.45µm, Bio-rad code n° 162-0114) at 100 volts for 1 hour using a Bio-rad Trans-blot system (code n°170-3930). Filter was blocked with PBS - 0.05% Tween 20 overnight at room temperature, before incubation with the mice sera containing the anti-BASB029 antibodies coming from both $AlPO_4$ and SBAS2 formulations. These sera are diluted 100 times in PBS - 0.05% Tween 20, and incubated on the nitrocellulose sheet for two hours at room temperature with gentle

shaking, using a mini-blotter system (Miniprotean, Bio-rad code n° 170-4017). After three repeated washing steps in PBS - 0.05% Tween 20 for 5 min., the nitrocellulose sheet is incubated at room temperature for 1hour under gentle shaking with the appropriate conjugate (biotinylated anti-mouse Ig antibodies from sheep, Amersham code n°RPN1001) diluted at 1/500 in the same washing buffer. The membrane is washed three times as previously, and incubated for 30 min with agitation using the streptavidin-peroxidase complex (Amersham code n°1051) diluted at 1/1000 in the washing buffer. After the last three repeated washing steps, the revelation occurs during the 20 min incubation time in a 50 ml solution containing 30 mg 4-chloro-1-naphtol (Sigma), 10ml methanol, 40 ml PBS, and 30µl of H_2O_2. The staining is stopped while washing the membrane several times in distillated water.

Results illustrated in Figures 4 and 5 show that all strains tested present the expected band around 65-70 kDa, and two other major bands around 55 and 90 kD, which are clearly related to this BASB029 protein (polymers, degradation products). This means that the BASB029 protein is probably expressed in most of, if not all, the NmB strains. In Figure 4, the recombinant BASB029 protein appears as four different proteins, two at the expected molecular weights of 65-70 Kda, and two others at very molecular heights (> 200 kDa) which are probably aggregates of recombinant BASB029 protein.

Example 4: Presence of anti-BASB029 antibodies in sera from convalescent patients.

In this test, convalescent sera have been tested by western-blotting for recognition of the purified recombinant BASB029 protein.

Briefly, 5µg of partially purified BASB029 NmB protein are put into a SDS-PAGE gradient gel (4-20%, Novex, code n°EC60252) for electrophoretic migration. Proteins are transferred to nitrocellulose sheet (0.45µm, Bio-rad code n° 162-0114) at 100 volts for 1 hour using a Bio-rad Trans-blot system (code n°170-3930). Afterwards, filter is blocked with PBS - 0.05 % Tween 20 overnight at room temperature, before incubation with the human sera. These sera are diluted 100 times in PBS - 0.05% Tween 20, and incubated on the nitrocellulose sheet for two hours at room temperature with gentle shaking, using a mini-blotter system (Miniprotean, Bio-rad code n° 170-4017). After three repeated washing steps in PBS - 0.05% Tween 20 for 5 min., the nitrocellulose sheet is incubated at room temperature for 1hour under gentle shaking with the appropriate conjugate (biotinylated anti-human Ig antibodies, from sheep, Amersham code n°RPN1003) diluted at 1/500 in the same washing buffer. The membrane is washed three times as previously, and incubated for 30 min with agitation using the streptavidin-peroxidase complex (Amersham code n°1051) diluted at 1/1000 in the washing buffer. After the last three repeated washing steps, the revelation occurs during the 20 min incubation time in a 50ml solution containing 30 mg 4-chloro-1-naphtol (Sigma), 10ml methanol, 40ml of ultra-pure water, and 30µl of H_2O_2. The staining is stopped while washing the membrane several times in distillated water.

Results are illustrated in Figures 6 and 7 which show that all 7 convalescents react against the rec. BASB029 protein at around 65 – 70 kDa, while the two upper bands (> 200 kDa) are also recognized by most of them, while with a weak reaction for few of them. Reactivities against the high MW proteins confirms that these two bands are clearly related to the BASB029 protein, which are probably under their aggregated forms. In part A of the Figures 6 and 7, the same reactions against these four bands are seen with the immunized mice sera. This clearly confirms that all these four bands are related to the recombinant BASB029 protein. Negative mice sera don't react with the recombinant protein as shown also in Fig 7.

Deposited materials

A deposit containing a *Neisseria meningitidis* Serogroup B strain has been deposited with the American Type Culture Collection (herein "ATCC") on June 22, 1997 and assigned deposit number 13090. The deposit was described as *Neisseria meningitidis* (Albrecht and Ghon) and is a freeze-dried, 1.5-2.9 kb insert library constructed from *N. meningitidis* isolate. The deposit is described in Int. Bull. Bacteriol. Nomencl. Taxon. 8: 1-15 (1958).

The *Neisseria meningitidis* strain deposit is referred to herein as "the deposited strain" or as "the DNA of the deposited strain."

The deposited strain contains the full length BASB029 gene. The sequence of the polynucleotides contained in the deposited strain, as well as the amino acid sequence of any polypeptide encoded thereby, are controlling in the event of any conflict with any description of sequences herein.

The deposit of the deposited strain has been made under the terms of the Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for Purposes of Patent Procedure. The strain will be irrevocably and without restriction or condition released to the public upon the issuance of a patent. The deposited strain is provided merely as convenience to those of skill in the art and is not an admission that a deposit is required for enablement, such as that required under 35 U.S.C. §112.

INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 55, line 1-22

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution

AMERICAN TYPE CULTURE COLLECTION

Address of depositary institution *(including postal code and country)*

10801 UNIVERSITY BLVD, MANASSAS, VIRGINIA 20110-2209, UNITED STATES OF AMERICA

Date of deposit	Accession Number
22 June 1997 (22/06/97)	13090

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

In respect of those designations where a European Patent is sought, a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European Patent or until the date on which the application has been refused or withdrawn, only by issue of such a sample to an expert nominated by the person requesting the sample.

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☐ This sheet was received with the international application	☐ This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

Form PCT/RO/134 (July 1998)

CLAIMS:

1. An isolated polypeptide comprising an amino acid sequence which has at least 85% identity to the amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4.

2. An isolated polypeptide as claimed in claim 1 in which the amino acid sequence has at least 95% identity to the amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4.

3. The polypeptide as claimed in claim 1 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4.

4. An isolated polypeptide of SEQ ID NO:2, SEQ ID NO:4.

5. An immunogenic fragment of the polypeptide as claimed in any one of claims 1 to 4 in which the immunogenic activity of said immunogenic fragment is substantially the same as the polypeptide of SEQ ID NO:2, SEQ ID NO:4.

6. An isolated polynucleotide comprising a nucleotide sequence encoding a polypeptide that has at least 85% identity to the amino acid sequence of SEQ ID NO:2, 4 over the entire length of SEQ ID NO:2, 4 respectively; or a nucleotide sequence complementary to said isolated polynucleotide.

7. An isolated polynucleotide comprising a nucleotide sequence that has at least 85% identity to a nucleotide sequence encoding a polypeptide of SEQ ID NO:2, 4 over the entire coding region; or a nucleotide sequence complementary to said isolated polynucleotide.

8. An isolated polynucleotide which comprises a nucleotide sequence which has at least 85% identity to that of SEQ ID NO:1, 3 over the entire length of SEQ ID NO:1, 3 respectively; or a nucleotide sequence complementary to said isolated polynucleotide.

9. The isolated polynucleotide as claimed in any one of claims 6 to 8 in which the identity is at least 95% to SEQ ID NO:1, 3.

10. An isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO:2, SEQ ID NO:4.

11. An isolated polynucleotide comprising the polynucleotide of SEQ ID NO:1, SEQ ID NO:3.

12. An isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO:2, SEQ ID NO:4 obtainable by screening an appropriate library under stringent hybridization conditions with a labeled probe having the sequence of SEQ ID NO:1, SEQ ID NO:3 or a fragment thereof.

13. An expression vector or a recombinant live microorganism comprising an isolated polynucleotide according to any one of claims 6 - 12.

14. A host cell comprising the expression vector of claim 13 or a subcellular fraction or a membrane of said host cell expressing an isolated polypeptide comprising an amino acid sequence that has at least 85% identity to the amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4.

15. A process for producing a polypeptide comprising an amino acid sequence that has at least 85% identity to the amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4 comprising culturing a host cell of claim 14 under conditions

sufficient for the production of said polypeptide and recovering the polypeptide from the culture medium.

16. A process for expressing a polynucleotide of any one of claims 6 – 12 comprising transforming a host cell with the expression vector comprising at least one of said polynucleotides and culturing said host cell under conditions sufficient for expression of any one of said polynucleotides.

17. A vaccine composition comprising an effective amount of the polypeptide of any one of claims 1 to 5 and a pharmaceutically acceptable carrier.

18. A vaccine composition comprising an effective amount of the polynucleotide of any one of claims 6 to 12 and a pharmaceutically effective carrier.

19. The vaccine composition according to either one of claims 17 or 18 wherein said composition comprises at least one other *Neisseria meningitidis* antigen.

20. An antibody immunospecific for the polypeptide or immunological fragment as claimed in any one of claims 1 to 5.

21. A method of diagnosing a *Neisseria meningitidis* infection, comprising identifying a polypeptide as claimed in any one of claims 1 - 5, or an antibody that is immunospecific for said polypeptide, present within a biological sample from an animal suspected of having such an infection.

22. Use of a composition comprising an immunologically effective amount of a polypeptide as claimed in any one of claims 1 – 5 in the preparation of a medicament for use in generating an immune response in an animal.

23. Use of a composition comprising an immunologically effective amount of a polynucleotide as claimed in any one of claims 6 - 12 in the preparation of a medicament for use in generating an immune response in an animal.

24. A therapeutic composition useful in treating humans with *Neisseria meningitidis* disease comprising at least one antibody directed against the polypeptide of claims 1 – 5 and a suitable pharmaceutical carrier.

Fig.1

Alignment of the BASB029 polynucleotide sequences.
Identity to SeqID No:1 is indicated by a dot, and a dash ("-") indicates a missing nucleotide.

```
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Seqid1:ATGAACAAAATATACCGCATCATTTGGAATAGTGCCCTCAATGCCTGGGT:50
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                 60          *         80          *        100
Seqid1:CGCCGTATCCGAGCTCACACGCAACCACACCAAACGCGCCTCCGCAACCG:100
Seqid3:.................................................:100

                  *        120          *        140          *
Seqid1:TGGCGACCGCCGTATTGGCGACACTGTTGTTTGCAACGGTTCAGGCGAGT:150
Seqid3:..AA.............................................A...:150

                 160          *        180          *        200
Seqid1:ACTACCGAT---------GACGACGATTTATATTTAGAACCCGTACAACG:191
Seqid3:G...A.A..GAAGAGCAA..A..A............C............:200

                  *        220          *        240          *
Seqid1:CACTGCTGTCGTGTTGAGCTTCCGTTCCGATAAAGAAGGCACGGGAGAAA:241
Seqid3:.....T..C........TAG..AA.........................:250

                 260          *        280          *        300
Seqid1:AAG---AAGTTACAGAAGATTCAAATTGGGGAGTATATTTCGACAAGAAA:288
Seqid3:...AAA....AGA....A.....G......C..........A..G.....:300

                  *        320          *        340          *
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Seqid3:...............A..GA..............................:350
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```
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Seqid1:AATCAAACAAAACACCAATGAAAACACCAATGCCAGTAGCTTCACCTACT:388
Seqid3:.....----------.G..CA.A.....:382

                *         420         *         440         *
Seqid1:CGCTGAAAAAAGACCTCACAGATCTGACCAGTGTTGGAACTGAAAAATTA:438
Seqid3:.....:432

                460         *         480         *         500
Seqid1:TCGTTTAGCGCAAACAGCAATAAAGTCAACATCACAAGCGACACCAAAGG:488
Seqid3:.....G.....:482

                *         520         *         540         *
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Seqid3:.....T.....G.....G...G.....:532

                560         *         580         *         600
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Seqid3:.C.....T.....:582

                *         620         *         640         *
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Seqid3:.....:632

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Seqid3:.....:682
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             1260          *        1280          *        1300
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Seqid3:.................................................:1282

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             1360          *        1380          *        1400
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Seqid3:.....G...........................................:1382

                  *        1420          *        1440          *
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Seqid3:G.........----.G..A...A..................A.G.A.....:1429
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                     *          1520          *          1540          *
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Seqid3:.....G...:1529

                1560          *          1580          *          1600
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Seqid3:.....:1579

                     *          1620          *          1640          *
Seqid1:CCGCAGGTCTGGTTCAGGCGTATCTGCCCGGCAAGAGTATGATGGCGATC:1638
Seqid3:.....T.....:1629

                1660          *          1680          *          1700
Seqid1:GGCGGCGGCACTTATCGCGGCGAAGCCGGTTATGCCATCGGCTACTCAAG:1688
Seqid3:.....C.....C...:1679

                     *          1720          *          1740          *
Seqid1:CATTTCCGACGGCGGAAATTGGATTATCAAAGGCACGGCTTCCGGCAATT:1738
Seqid3:T.....:1729

                1760          *          1780          *
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Seqid3:.....:1776
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Fig.2

Alignment of the BASB029 polypeptide sequences.
Identity to SeqID No:2 is indicated by a dot, and a dash ("-") indicates a missing amino acid.

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                  *         20          *         40          *
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Seqid4:ANNEEQEE....D.....VA..IVN.....K.E.....A....E.:100

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```

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```
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Seqid4:..................................................:444

                  460         *       480         *       500
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Seqid4:....................GD-.......KD..................:493

                  *         520       *         540       *
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Seqid4:...............R..................................:543

                  560         *       580         *
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Seqid4:................................................:591
```

Fig.3 Expression and purification of recombinant BASB029 in E. coli.

A substatially purified BASB029 protein fraction (more than 80%) was seperated on a 4-20% gradient polyacrylamide gel (NOVEX) under PAGE-SDS conditions in parallel to a protein molecular weight marker. Gels were either stained with Coomassie Blue R250 (lane 1) or analyzed by western blot using an anti-(His5) monoclonal antibody (lane 2).

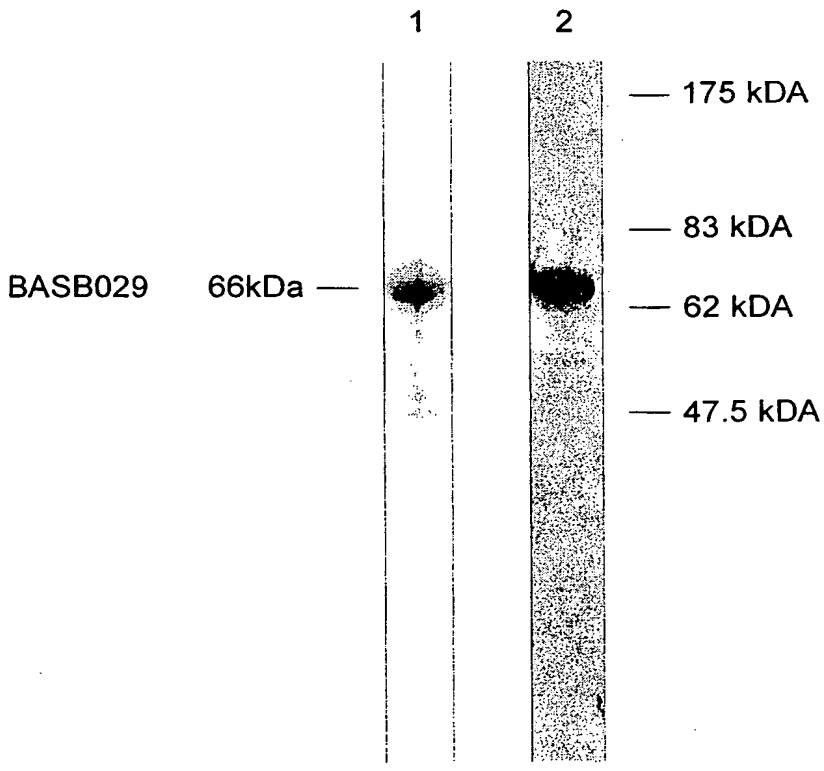


Fig.4

Recognition of the BASB029 protein on several NmB strains with BASB029 immunized mice sera

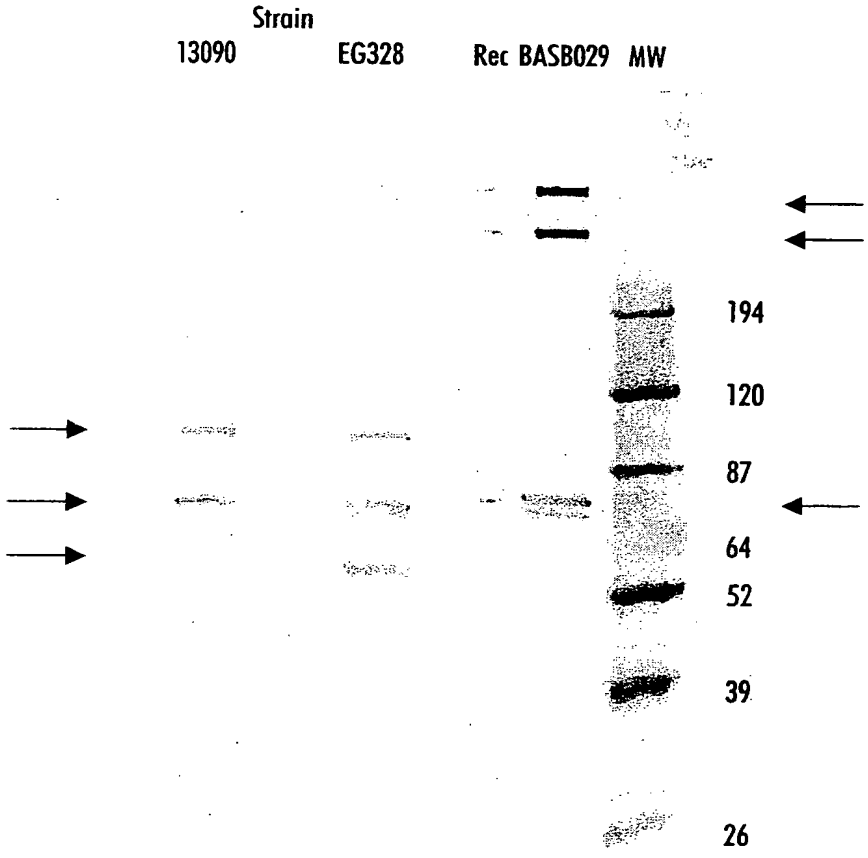


Fig.5

Recognition of the BASB029 protein on several NmB strains with BASB029 immunized mice sera

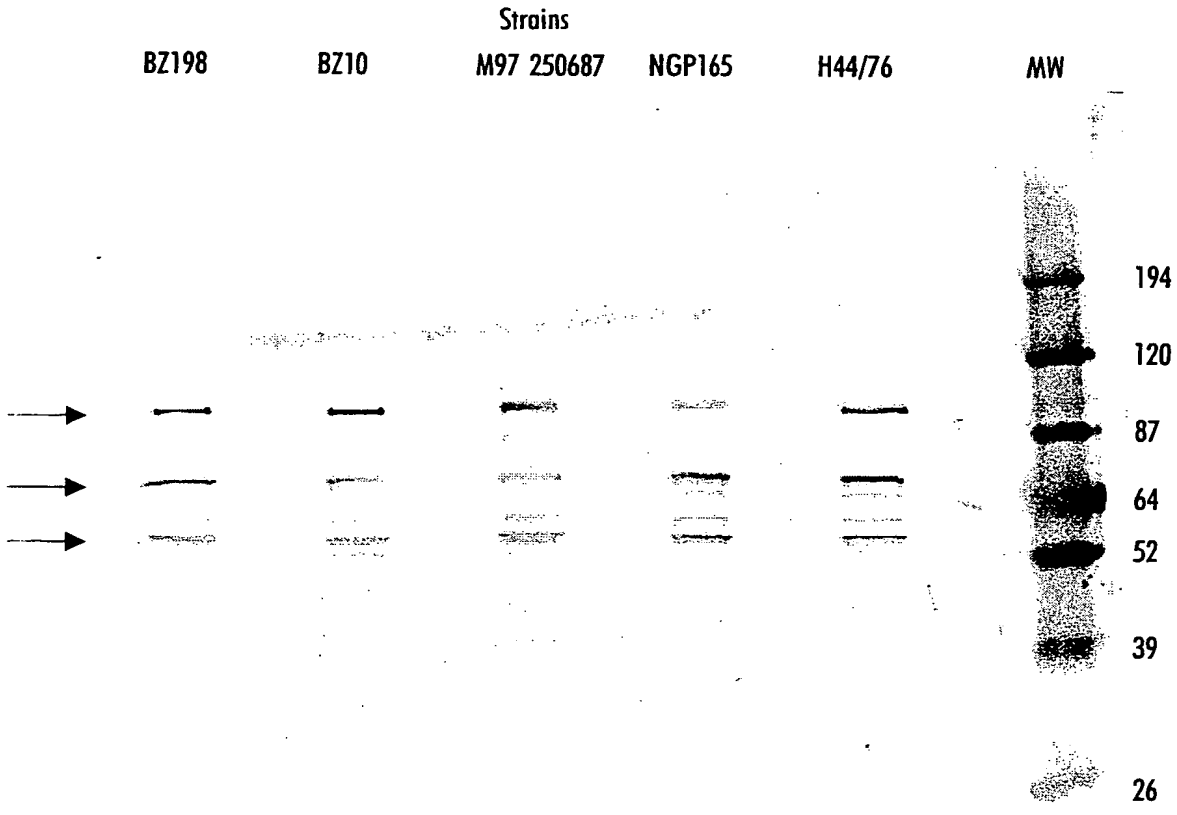


Fig.6

Anti-BASB029 antibodies in convalescent sera (part B) and in immunized mice (part A).

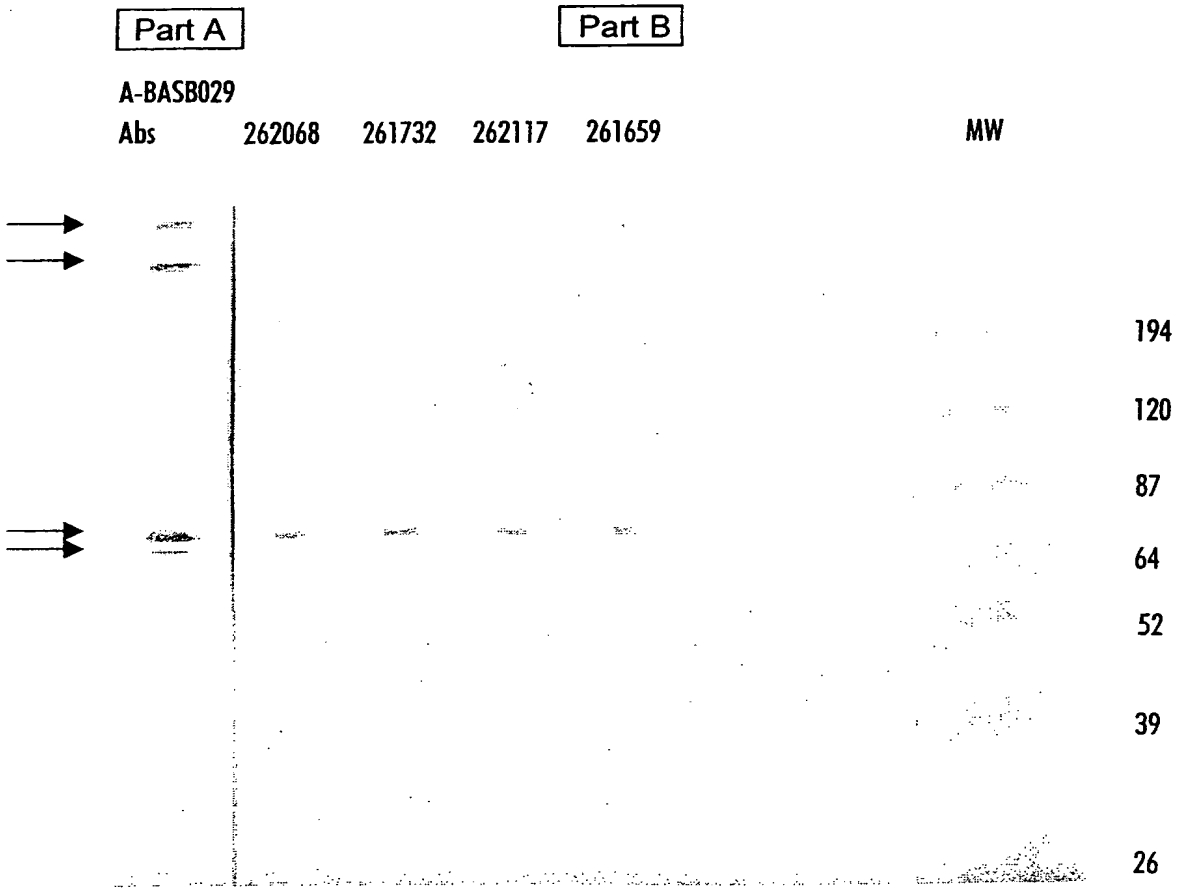
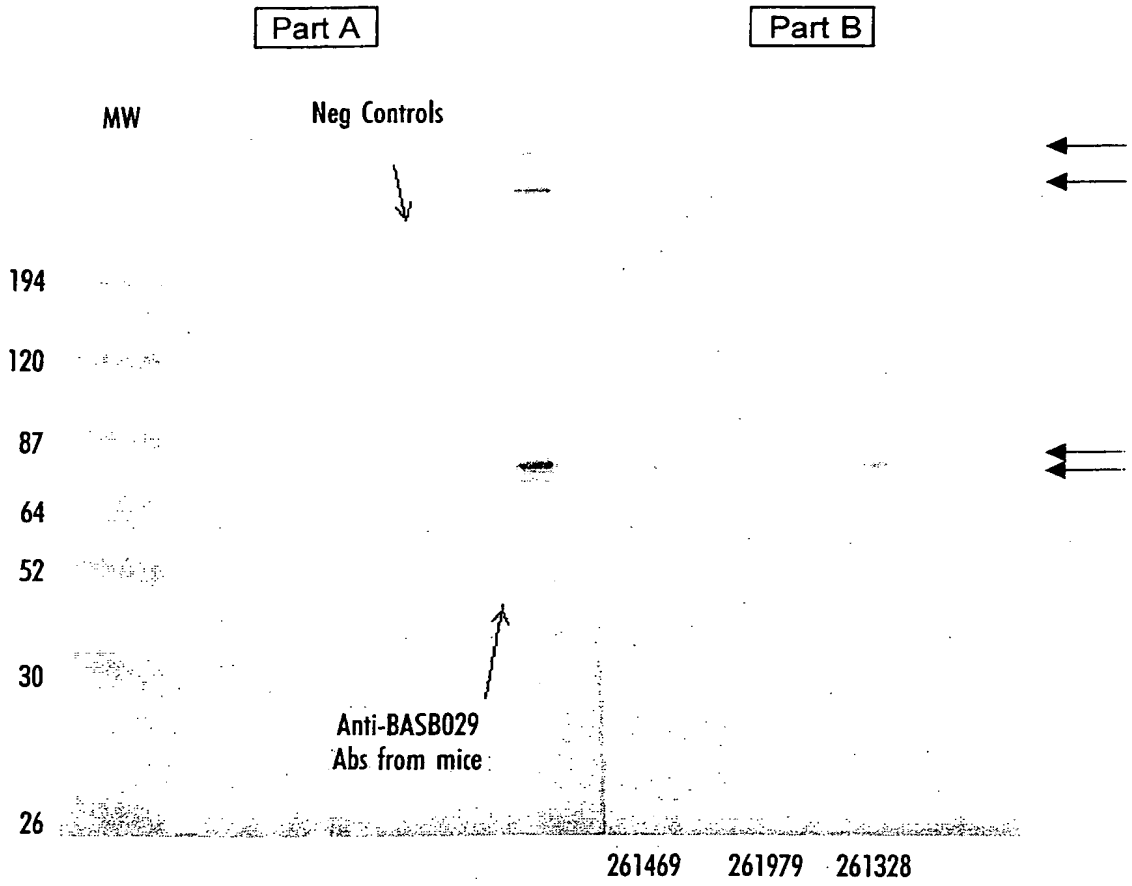


Fig.7

Anti-BASB029 antibodies in convalescent sera (part B) and in immunized mice (part A).



SEQUENCE LISTING

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35 40 45
Ala Ser Thr Thr Asp Asp Asp Asp Leu Tyr Leu Glu Pro Val Gln Arg
50 55 60
Thr Ala Val Val Leu Ser Phe Arg Ser Asp Lys Glu Gly Thr Gly Glu
65 70 75 80
Lys Glu Val Thr Glu Asp Ser Asn Trp Gly Val Tyr Phe Asp Lys Lys
85 90 95
Gly Val Leu Thr Ala Gly Thr Ile Thr Leu Lys Ala Gly Asp Asn Leu
100 105 110
Lys Ile Lys Gln Asn Thr Asn Glu Asn Thr Asn Ala Ser Ser Phe Thr
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Tyr Ser Leu Lys Lys Asp Leu Thr Asp Leu Thr Ser Val Gly Thr Glu
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Lys Leu Ser Phe Ser Ala Asn Ser Asn Lys Val Asn Ile Thr Ser Asp
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Thr Lys Gly Leu Asn Phe Ala Lys Lys Thr Ala Glu Thr Asn Gly Asp

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Trp Asn Ile Lys Gly Val Lys Pro Gly Thr Thr Ala Ser Asp Asn Val
225                 230                 235                 240
Asp Phe Val Arg Thr Tyr Asp Thr Val Glu Phe Leu Ser Ala Asp Thr
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Lys Thr Thr Thr Val Asn Val Glu Ser Lys Asp Asn Gly Lys Arg Thr
            260                 265                 270
Glu Val Lys Ile Gly Ala Lys Thr Ser Val Ile Lys Glu Lys Asp Gly
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Lys Leu Val Thr Gly Lys Asp Lys Gly Glu Asn Asp Ser Ser Thr Asp
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50 55 60
Val Gln Arg Thr Val Ala Val Leu Ile Val Asn Ser Asp Lys Glu Gly
65 70 75 80
Thr Gly Glu Lys Glu Lys Val Glu Glu Asn Ser Asp Trp Ala Val Tyr
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Val Thr Gly Lys Asp Lys Gly Glu Asn Gly Ser Ser Thr Asp Glu Gly
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Glu Gly Leu Val Thr Ala Lys Glu Val Ile Asp Ala Val Asn Lys Ala
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            340                 345                 350
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Thr Val Met Tyr Asp Val Asn Val Gly Asp Ala Leu Asn Val Asn Gln
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Ser Gly Lys Val Ile Ser Gly Asn Val Ser Pro Ser Lys Gly Lys Met
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Asp Glu Thr Val Asn Ile Asn Ala Gly Asn Asn Ile Glu Ile Thr Arg
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Asn Gly Lys Asn Ile Asp Ile Ala Thr Ser Met Thr Pro Gln Phe Ser
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Gly Leu Val Gln Ala Tyr Leu Pro Gly Lys Ser Met Met Ala Ile Gly
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BASB029 Polynucleotide and Polypeptide Sequences

SEQ ID NO:1

Neisseria meningitidis BASB029 polynucleotide sequence

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AAAGAAGTTACAGAAGATTCAAATTGGGGAGTATATTTCGACAAGAAAGGAGTACTAACA
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ACCAAAGGCTTGAATTTCGCGAAAAAAACGGCTGAGACCAACGGCGACACCACGGTTCAT
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GTAACCAACGACAACGTTACCGATGACGAGAAAAAACGTGCGGCAAGCGTTAAAGACGTA
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CGCGGCCATTTCGGTGCTTCCGCATCTGTCGGTTATCAGTGGTAA
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SEQ ID NO:2

Neisseria meningitidis BASB029 polypeptide sequence deduced from the polynucleotide sequence of SEQ ID NO:1

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MNKIYRIIWNSALNAWVAVSELTRNHTKRASATVATAVLATLLFATVQASTTDDDDLYLE
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NTNASSFTYSLKKDLTDLTSVGTEKLSFSANSNKVNITSDTKGLNFAKKTAETNGDTTVH
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DFVRTYDTVEFLSADTKTTTVNVESKDNGKRTEVKIGAKTSVIKEKDGKLVTGKDKGEND
SSTDKGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNVTFASGKGTTA
TVSKDDQGNITVMYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGNVSPSKGKMDE
TVNINAGNNIEITRNGKNIDIATSMTPQFSSVSLGAGADAPTLSVDDEGALNVGSKDANK
PVRITNVAPGVKEGDVTNVAQLKGVAQNLNNHIDNVDGNARAGIAQAIATAGLVQAYLPG
KSMMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASASVGYQW

SEQ ID NO:3

***Neisseria meningitidis* BASB029 polynucleotide sequence from strain H44/76**

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TATTTAGACCCCGTACAACGCACTGTTGCCGTGTTGATAGTCAATTCCGATAAAGAAGGC
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ACTTCGATGACCCCGCAGTTTTCCAGCGTTTCGCTCGGCGCGGGGGCGGATGCGCCCACT
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TTCGGTGCTTCCGCATCTGTCGGTTATCAGTGGTAA

SEQ ID NO:4

Neisseria meningitidis BASB029 polypeptide sequence deduced from the polynucleotide sequence of SEQ ID NO:3

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GIGSTLTDTLLNTGATTNVTNDNVTDDEKKRAASVKDVLNAGWNIKGVKPGTTASDNVDF
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ITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIATAGLVQAYLPGKSM
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SEQ ID NO:5

GGG GCA TAT GAA CAA AAT ATA CCG CAT CAT TTG GAA

SEQ ID NO:6

GGG GCT CGA GCC ACT GAT AAC CGA CAG ATG CGG A

INTERNATIONAL SEARCH REPORT

Intn ional Application No
PCT/EP 99/03255

A. CLASSIFICATION OF SUBJECT MATTER
IPC7 C12N15/31 C07K14/22 C07K16/12 A61K39/095 A61K48/00
C12N5/10 C12Q1/68 G01N33/53 A61P31/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K A61K C12Q G01N A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ST GEME JW 3RD, CUTTER D, BARENKAMP SJ : "Characterization of the genetic locus encoding Haemophilus influenzae type b surface fibrils" J BACTERIOL. , vol. 178, no. 21, November 1996 (1996-11), pages 6281-6287, XP000863110 the whole document	1
A	EP 0 301 992 A (NACIONAL DE BIOPREPARADOS CENT) 1 February 1989 (1989-02-01) the whole document	1
A	WO 93 06861 A (PASTEUR MERIEUX SERUMS VACC) 15 April 1993 (1993-04-15) the whole document	17,22-24

-/—

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 December 1999	11/01/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Chambonnet, F

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inte ional Application No
PCT/EP 99/03255

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 97 26359 A (ALVAREZ ACOSTA ANABEL ;GUILLEN NIETO GERARDO ENRIQUE (CU); NAZABAL) 24 July 1997 (1997-07-24) the whole document	17,22-24
E	WO 99 31132 A (JENNINGS MICHAEL PAUL ;PEAK IAN RICHARD ANSELM (AU); UNIV QUEENSLA) 24 June 1999 (1999-06-24) the whole document	1-24

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int tional Application No
PCT/EP 99/03255

Patent document cited in search report	Publication date	Patent family member( )	Publication date
EP 0301992 A	01-02-1989	AT 122893 T	15-06-1995
		AU 615461 B	03-10-1991
		AU 2031288 A	25-05-1989
		AU 5319794 A	24-03-1994
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WO 9726359 A	24-07-1997	AU 1539697 A	11-08-1997
		BR 9704641 A	09-06-1998
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		CZ 9702910 A	11-11-1998
		EP 0816506 A	07-01-1998
		HU 9800730 A	28-07-1998
		JP 11503617 T	30-03-1999
WO 9931132 A	24-06-1999	AU 1649599 A	05-07-1999

BG

09/700293

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
FB/BM45321		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 99/03255	07/05/1999	13/05/1998
Applicant		
SMITHKLINE BEECHAM BIOLOGICALS S.A.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [X] contained in the international application in written form.

   [X] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established by this Authority to read as follows:

   BASB029POLYNUCLEOTIDE(S) AND POLYPEPTIDES FROM NEISSERIA MENINGITIS

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 8

   [ ] as suggested by the applicant.

   [X] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 99/03255

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WO 97 26359 A (ALVAREZ ACOSTA ANABEL ;GUILLEN NIETO GERARDO ENRIQUE (CU); NAZABAL) 24 July 1997 (1997-07-24) the whole document	17,22-24
E	WO 99 31132 A (JENNINGS MICHAEL PAUL ;PEAK IAN RICHARD ANSELM (AU); UNIV QUEENSLA) 24 June 1999 (1999-06-24) the whole document	1-24

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International Application No
PCT/EP 99/03255

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 99/03255

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/31 C07K14/22 C07K16/12 A61K39/095 A61K48/00
C12N5/10 C12Q1/68 G01N33/53 A61P31/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K A61K C12Q G01N A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ST GEME JW 3RD, CUTTER D, BARENKAMP SJ : "Characterization of the genetic locus encoding Haemophilus influenzae type b surface fibrils" J BACTERIOL. , vol. 178, no. 21, November 1996 (1996-11), pages 6281-6287, XP000863110 the whole document ---	1
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[X] Further documents are listed in the continuation of box C.

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Date of the actual completion of the international search	Date of mailing of the international search report
17 December 1999	11/01/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Chambonnet, F

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(71) Applicant *(for all designated States except US)*: SMITHKLINE BEECHAM BIOLOGICALS S.A. [BE/BE]; Rue de l'Institut 89, B–1330 Rixensart (BE).

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(74) Agent: TYRRELL, Arthur, William, Russell; SmithKline Beecham, Two New Horizons Court, Brentford, Middlesex TW8 9EP (GB).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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6 April 2000 (06.04.00)

(54) Title: BASB029 POLYNUCLEOTIDE(S) AND POLYPEPTIDES FROM *NEISSERIA MENINGITIDIS*

(57) Abstract

The invention provides BASB029 polypeptides and polynucleotides encoding BASB029 polypeptides and methods for producing such polypeptides by recombinant techniques. Also provided are diagnostic, prophylactic and therapeutic uses.

INTERNATIONAL SEARCH REPORT

Inte ional Application No
PCT/EP 99/03255

A. CLASSIFICATION OF SUBJECT MATTER
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